140 | II

COMMONWEALTH MYCOLOGICAL INSTITUTE
1 MAR 1966

MEDEDEELINGEN

VAN HET

PHYTOPATHOLOGISCH LABORATORIUM
„WILLIE COMMELIN SCHOLTEN"
BAARN.

XI. MEI 1927.

1. ROOT ROTS CAUSED BY PHYCOMYCETES, by Dr. C. J. BUISMAN.

2. BRUNCHORSTIA DESTRUENS ERIKSS. AUF PINUS LARICIO VAR. CORSICANA UND IHRE REINKULTUR, von A. VAN LUIJK.

3. PILZKULTUREN IN FEUCHTEN KAMMERN, von A. VAN LUIJK.

4. DIE BOTRYTIS-KRANKHEIT DER PÄONIEN, von F. H. VAN BEYMA THOE KINGMA.

HOLLANDIA-DRUKKERIJ, BAARN.

ROOT ROTS CAUSED BY PHYCOMYCETES

BY

Dr. C. J. BUISMAN

INTRODUCTION

These investigations were originally undertaken with the idea of adding something to our knowledge of the root rot of peas caused by *Phycomycetes*. In America several publications have appeared about it during the last few years, while very little attention has up to now been given to it in Europe. It would, for instance, be of importance to investigate if the dangerous american root parasite of peas, *Aphanomyces euteiches*, Drechsler, is also present in diseased pea roots in this country and if it is the cause of rootrot.

As it was necessary that I first gained some experience with Phycomycetes, I examined several different diseased plants which were given to me as probably being infected by a fungus of that group.

I will also discuss some of these diseases which up till now were still unknown.

This applies in the first instance to the disease of the roots of the *Calla-lily* which causes a considerable loss to the *Calla*-nurseries in this country and has been thought to be due to Nematodes. It happened that diseased *Chrysanthemum* cuttings, which had suddenly stopped growing, were also sent to the laboratory at the time.

In the course of time I could examine different rots of cuttings, several of which I described in this publication.

While examining the different pea roots it struck me, that *Asterocystis radicis* de Wildeman, the wellknown parasite on flax roots which is taken to be the cause of „brand" = fire in flax, is often found on them. In order to be able to recognize this *Chytridiacea* and not to mistake it for oogonia of *Phycomycetes* I also examined roots of diseased flax. In doing so I discovered a

Phycomycete which is apparently parasitic on the flax roots, and which I have therefore also described here.

As most of these plants had to be cultivated in vegetable mould it was necessary to know something about the Phycomycetes which inhabit humus. If our soil proved to be strongly infected by them, inoculation experiments in it would be worthless. Some more observations about the different Phycomycetes which I isolated from seedlings grown in our vegetable mould will also have to be considered.

This work was carried out more along phytopathological than along morphological lines. I could only make a choice out of all the isolations which were at my disposition.

A thorough treatment of the genera *Pythium* and *Phytophthora* would lead me too far, so I am restricting myself to some considerations about the classification of the group to which they belong, and about some related genera.

CHAPTER I

TAXONOMY

§ 1. *The classification in the Phytophthoreae*

In the year 1881 de Bary (4) already wrote about the classification of the group *Phytophthoreae*, in connection with the genera *Pythium* and *Phytophthora*. He showed that there is far too little difference between these genera to justify placing them in different families as Pringsheim had done (Pythium with the *Saprolegniaceae*, Phytophthora with the *Peronosporaceae*). De Bary pointed out, that there is really no other difference between Pythium and Phytophthora than the way in which the zoospores are formed. In the genus Pythium the protoplasm from the sporangium flows into a vesicle in which the zoospores are then formed, whereas in the genus Phytophthora the zoospores are formed in the sporangium itself.

Butler's monograph (10) about the genus Pythium is the best source of data at present. He agrees with de Bary and also sees no other difference between Pythium and Phytophthora. He includes both genera in the Peronosporaceae.

A detailed discussion about this question is found in a chapter from ,,Generic concepts in the *Pythiaceae* and *Blastocladiaceae*" by Fitzpatrick (14), i. e. *The Pythium — Phytophthora Problem.*

This shows, that an absolute separation between Pythium and Phytophthora is theoretically impossible, as it has become evident that the traditional characteristic which separates Pythium from Phytophthora, namely the formation of zoospores in a vesicle, in-

stead of in the sporangium, is also found amongst different Phytophthora species. —

But in literature we find other species which belong to the group of Pythium and Phytophthora. How are they related to Pythium and Phytophthora?

In the first place the genus *Pythiacystis*. Barrett (2), E. Smith and R. Smith (33) and Leonian (20) pointed out, that a separation between Pythiacystis and Phytophthora cannot be carried through. Smith and Smith even isolated forms, which showed a transition from Pythiacystis to *Phytophthora cactorum*. Leonian has now definitely dropped the genus Pythiacystis and uses the name *Phytophthora citrophthora*.

Dufrénoy (13) includes the genus *Blepharospora* Petri in the homogenous group of Phytophthora, Pythium and Pythiacystis (which he, like Fitzpatrick (14), separates from the real Peronosporaceae with aerial conidia and which are obligate parasites). Fitzpatrick further regards the genus *Pythiogeton* v. Minden as a representative of this group.

According to my opinion, *Pythiomorpha* Petersen is another genus which belongs to it.

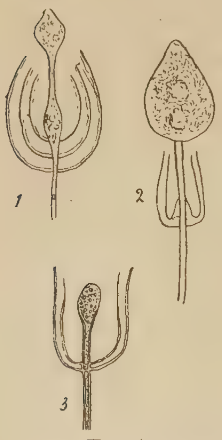

Fig. 1
1 and 2: Zoosporangium of Pythiomorpha gonapodyides after Miss Kanouse (high power).
3: Zoosporangium of Blepharospora cambivora after Petri. ± 333 ×

The question is, whether there are sufficient reasons for letting these genera exist next to Pythium and Phytophthora. I do not think, that there ought to be any difficulty in letting the genus Pythiogeton remain, as it is sufficiently characterized by its asymetrical sporangia. — It is a different question with Blepharospora and Pythiomorpha however. —

According to my opinion these genera, each with one representative, *Blepharospora cambivora* Petri and *Pythiomorpha gonapodyides* Petersen, should be included in the genus *Phytophthora*. The reasons for this I will give below.

I do not think that Petri (27, 28, 29) succeeded in proving definitely that his Blepharospora is not a Phytophthora.

His principal arguments in favour of making a new genus are:

1. that Blepharospora does not produce sporangia on the surface of the infected parts of the plants or on solid culture media, but only in a mineral salt solution.
2. that Blepharospora possesses long, unbranched sporangiophores ending in *one* sporangium.

It will become obvious below that representatives of the genus Phytophthora also show similar characteristics.

Blepharospora is related to *P. cactorum, P. fagi* and *P.syringae,* according to Petri, because it possesses paragynous antheridia. Although Pethybridge does no longer consider the possession of paragynous or amphigynous antheridia a reason for subdividing the genus Phytophthora, as both kinds of antheridia sometimes occur in one form, Petri still maintains that this subdivision should remain.

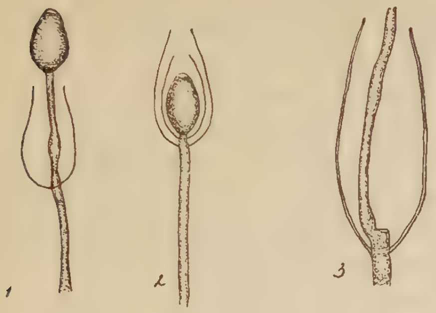

Fig. 2
Zoosporangia of Phytophthora cinnamomi after Rands.
1 and 2 ± 237 ×, 3 370 ×

Fig. 3
Zoosporangia of Phytophthora cryptogea after Pethybridge. 340 ×

A Phytophthora would then belong to the one or to the other group, as it more regularly produces amphigynous or paragynous antheridia. In this case Blepharospora would then belong close to Phytophthora cactorum. I do not attach very much importance to this production of antheridia, as Blepharospora forms oogonia only in the tissues of the infected chestnut-tree, and it is therefore

difficult to determine the position of the antheridium. The characteristics show that Blepharospora is more closely related to *Phytophthora cryptogea* and *P. cinnamomi.*

These characteristics are exactly the same as the ones, Petri uses to separate Blepharospora from the genus Phytophthora, namely the difficulty in forming sporangia and the unbranched sporangiophores.

Phytophthora cryptogea and P. cinnamomi also rarely produce sporangia and possess little branched conidiophores. Further more these two genera also have another characteristic in which they correspond with Blepharospora. The sporangiophore grows into the sporangium after its contents have been discharged. The sporangia are also rounded off above, without a definite papilla being formed, exactly as is the case with Blepharospora.

Petri separates Blepharospora from the genus Pythium because the zoospores are already formed in the sporangium, the same reason for which Phytophthora is separated from Pythium.

I therefore think that it is absolutely unjustified to let the genus Blepharospora exist next to the genus Phytophthora.

The same counts for the genus *Pythiomorpha* established in 1910 by Petersen (25). Von Minden (22) already pointed out that Petersen does not express himself about the reasons which induced him to form even a new family, Pythiomorphaceae, but he thinks Petersen is right. Pythiomorpha is again separated from Pythium, because the zoospores are directly formed in the sporangium instead of in a vesicle. The occurrence on loose twigs and fruit in water is not a special characteristic of the genus Phytophthora, but this is no reason for excluding Pythiomorpha from the genus Phytophthora, as it is known (see Dufrénoy) (13) that Phytophthora's may lead a saprophytic life.

In the detailed description of Pythiomorpha gonapodyides, the only existing species of the genus Pythiomorpha, by Miss Kanouse (19) there is no characteristic to be found which should separate Pythiomorpha from the Phytophthora's. Petersen emphasized the fact that the mycelium is coloured purple by chlorojodide of zinc, but when I applied the same method to Blepharospora, it gave the same results.

Pythiomorpha also shows the sporangiophores which grow into

the sporangium after the swarmspores have been set free and sporangia and oogonia are formed with difficulty just as in Blepharospora.

I think that I have now shown that the genera Blepharospora and Pythiomorpha should be included in the genus Phytophthora, so that we may speak of *Phytophthora cambivora* and *Phytophthora gonapodyides*. Then except Pythiogeton, only the genera Pythium and Phytophthora remain, as Pythiacystis has already been included in the genus Phytophthora.

According to my opinion it would be of no practical importance to unite these two genera into one. The name Phytophthora is so universally used that it would be confusing to drop it in favour of the (older) name Pythium.

It is enough to state, as other investigators have already done, that theoretically no sharp line can be drawn between Pythium and Phytophthora. In practice it is usually possible to determine if the form which is examined belongs to the „type" Phytophthora or to the „type" Pythium.

It becomes more difficult when no swarmspores are produced as in the case of *Pythium splendens* Braun. Braun (8) does not say if this fungus might perhaps be a Phytophthora. But as the conidia of this form are all round instead of possessing the pear-shaped aspect of most Phytophthora-sporangia, it was only reasonable to speak of a Pythium instead of a Phytophthora, although the shape of the spores is not an official difference. The same will probably happen in other cases where difficulties are met with in connection with the production of swarmspores.

§ 2. The classification in the genera Phytophthora and Pythium

Few genera have been studied so often as Phytophthora, and yet the classification is far from being settled.

At present it is very difficult and sometimes nearly impossible, to identify a Phytophthora. The different species of this genus are so much alike, that it is nearly impossible to determine which species one is dealing with. From the old species of de Bary, *P. omnivora*, the four species *P. fagi*, *P. cactorum*, *P. syringae* and *P. faberi* have since been separated. Leonian (20) now states

that there is no difference between *P. fagi* and *P. cactorum*, and eliminates *P. fagi*.

There are many other difficulties in the same way. It was first tried to settle these questions along purely morphological lines, afterwards physiological methods were tried for the same purpose.

Rosenbaum (32) wrote a very accurate study in which he gives a detailed morphological account of the different species. He finally separates them by means of several characteristics, for instance the difference in size of the zoosporangia and oogonia as is indicated by curves.

But this is a very difficult method to apply in practice. This publication of Rosenbaum is however still a very valuable acquisition to the Phytophthora literature, especially as the species are studied on different culture-media. Leonian's research was meant to give a solution of the problem along physiological lines. He declares, specially in connection with his investigations about the mutations of *Phytophthora parasitica rhei*, that the genus may not be classified according to the size of the sporangia, the presence or absence of oogonia, other characteristics which appear in culture, and pathogenicity.

In practice there are however many objections against his „physiological key". Leonian himself also realizes that it is very difficult to cultivate fungi under exactly the same physiological circumstances in different parts of the world.

It therefore becomes nearly impossible to split the genus up into several divisions and to draw a sharp line round each. The matter has become more complicated by the publications of Ashby (1) and Gadd (15).

They demonstrated, that heterothallism is found in the species *Phytophthora faberi*. This could also show, that different species of Phytophthora may form hybrids in this way, and as transitions between the different forms will then of course originate, a sharp separation between them would become absolutely impossible.

According to my opinion, the morphological differences between the species must for the present first be determined and then physiological characteristics may be added, so that we may get a system which will be useful in practice. The more so, as it is

easier to identify by means of morphological characteristics, than by the round about physiological method.

In the genus Pythium things are not so complicated as with Phytophthora. Still it is not at all easy to determine if a special isolation belongs to the otherwise wellknown *Pythium de Baryanum* or not.

Even Drechsler (12) who is writing a monograph about the genus Pythium speaks of „species" or „forms" of the *de Baryanum type*. I think that we must now be specially careful not to give too strict definitions of the species, as the *Phycomycetes* are very variable forms. Some species of Pythium for instance show a different tendency towards forming oogonia or sporangia. De Bary (4) already noticed this in 1881 with *P. megalacanthum*. I possess only one isolation of this species which forms only oogonia and no sporangia.

The same is the case with an undescribed Pythium I isolated from various plants. The one isolation forms sporangia and swarm-spores, although only few, the other nearly purely oogonia.

Nothing further is known about the sexuality of these forms. — Perhaps the many forms one meets without oogonia show that heterothallism exists as in *P. faberi*, or perhaps we have here a strong tendency towards production of asexual spores. This appears to be the case with *P. de Baryanum var. geranii* Braun (8) which produced a few oogonia in culture only after 14 months which even then often degenerated.

I also came across this form when isolating, but never noticed any oogonia.

Some uncertainty therefore also exists where the genus Pythium is concerned.

I never noticed anything of biological races adapted to certain hosts. According to Drechsler however, some forms of Pythium de Baryanum are more pathogenic than other forms of the same species.

This again shows the great variability which exists, also in connection which physiological characteristics. It is therefore advisable not to give too strict a definition of the different species.

CHAPTER II

EXPERIMENTAL METHODS

In making isolations from infected roots of the different hosts I proceeded as follows:

The diseased roots were layed out on agarplates without being disinfected, as this handling would have killed the delicate mycelium of the *Phycomycetes*. At first I always used cherry-agar for the isolations as it is rather acid and therefore arrests growth of bacteria. Afterwards I found that oatmeal-agar is a more desirable medium. Although it is more favourable for bacterial growth, the Phycomycetes grow better on it than on cherry-agar.

It is very difficult to keep the cultures absolutely free from bacteria as they adhere to the hyphae and are therefore difficult to remove. The best way is to cultivate the fungi on agarplates and to transfer them as often as possible.

I usually used oatmeal-agar for keeping them in culture. On cherry-agar they mostly also produce a woolly growth of mycelium but few oogonia are formed. *Pythium de Baryanum* however grows well on cherry-agar while *P. megalacanthum* does not grow at all.

On cornmeal-agar many oogonia are also formed, but this medium is even more favourable for the growth of bacteria and therefore not so suitable.

Swarmsporangia are, on a whole, seldom produced on agar media. In order to obtain them I adopted the method used by Petri for *Blepharospora* cambivora.

I mostly used the receipt as given by him:

0,400 gr. $Ca(NO_3)_2$
0,150 gr. KH_2PO_4
0,150 gr. $MgSO_4$
0,060 gr. KCl.
1000 gr. distilled water.

I cultivated a tuft of the mycelium from agarcultures for a few days in a flask, in which a thin layer of this solution had been brought. Hardly any growth of mycelium took place but apparently this anorganic solution served as a stimulus for the production of sporangia. The greatest number of sporangia were however formed when the bit of mycelium was afterwards transferred from the solution to a watchglass with distilled water. Sometimes the zoospores appeared directly; in other cases the water had to be refreshed first, and zoospores only appeared afterwards, for instance after one day. This method was successful with several kinds of Phytophthora-and Pythium-species which had at first hardly produced any zoospores, or none at all.

Even species which had been isolated years ago and had since then been kept in culture at the „Centraal Bureau voor Schimmelcultures", could be induced to form zoospores when treated in this way. With Aphanomyces euteiches I also obtained the same results.

It is a pity that the results are not always so satisfactory: *Pythium splendens* for instance, cannot be induced to form zoospores even by this method. In other cases there are so few that it is impossible to trace their development.

For inoculation material I used some strains which I had isolated from diseased plants, and some species which were kept in culture at the „Centraal Bureau v. Schimmelcultures". I never found any indications that the pathogenicity of these species had suffered by remaining in culture for so long. *Pythium complectens* from Braun, *Pythium splendens* from Leonian, and *P. de Baryanum* from Hartley which had been cultivated for some time, were still very virulent.

I will enter more fully into the methods of infection when I describe the experiments. The main point is that I often used watercultures in which the fungus was brought (see chapters on Calla-lily, peas and flax). As for the cuttings, their lower ends

were covered with the inoculation-material and then placed in sterilized sand.

Whenever I used vegetable mould for growing peas in, I sterilized it before use, because of the many *Phycomycetes* occurring in humus. (See chapter on Occurrence of Pythium in vegetable mould).

CHAPTER III

ROOT ROT OF THE CALLA-LILY

§ 1. Occurrence of the disease

Large hothouses with Calla-lilies are found on several commercial nurseries in our country. It is a very profitable culture as the flowers are very valuable and as each hothouse may yield some thousands of flowers.

The plants are treated in the following manner:

In spring they are put in the open, so that they may dry out and pass through a period of rest. After this period the corms are taken out of the pots, cleaned and planted again in fresh pots. They are left outside for another little while (not dry this time) and put in the hothouses at the beginning of winter. They start flowering in about December. In the last few years it often happened at several nurseries that the plants started to fade in December. The outer leaves then showed a yellow discoloration, which slowly spread over the surface until the whole leaf had turned yellow. The same happens to the other leaves until there are only a few left. The flowers are few and small so that the yield of a hothouse with diseased plants is very small. The disease is not very acute as new leaves are still being formed and the plant is not killed, but it remains ailing.

A diseased plant is easily withdrawn from the pots as the roots are easily broken off, and only small rotten pieces still adhere to the corm. The corm itself is sometimes also full of holes and grooves. If the plant is very carefully taken out of the pot with soil and

all, the roots appear to be badly infected. They have a glassy and drooping appearance, especially striking with the thicker roots. A reddish discoloration is also often found.

If the plants are treated in the usual way, e. g. if they pass through a period of rest and are then changed to other pots, there is at first no evidence of the disease. But, about the flowering season the symptoms appear again. It stands to reason that the corms suffer badly from such repeated occurrence of the disease.

§ 2. Microscopical examination of the roots and isolation of the pathogenic organism

One receives the same confused impression when examining the infected roots of the Calla-lily as when examining other diseased roots. First one notices the many *Nematodes*. The disease had at first been ascribed to them, but as most of them belonged to the not-parasitical kind *Rhabditis brevispina,* which lives in all kinds of decaying portions of plants, their presence was still no proof that they are indeed the cause of the disease.

Besides these Nematodes different kinds of soil-fungi are found in the roots. *Thielavia basicola* was occasionally found and also often oogonia of *Phycomycetes*. It was tried to find the cause by laying out small pieces of infected roots on agarplates. As the roots were so delicate, it was of course impossible to sterilize them first. This proved to be a great objection as all kinds of fast growing soil-fungi and saprophytes, which immediately attack the decaying roots, now appeared in my cultures. The isolations therefore only yielded common fungi such as *Pythium*-species and Botrytis. They are just ordinary inhabitants of vegetable mould and it was improbable that they would be the cause of the Calla-lily disease, which appeared relatively suddenly. After several unsuccessful attempts I gave up this method of isolating the pathogenic organism.

In the nurseries it had become evident that the disease also appears when the corms were dried and planted in absolutely fresh soil. This would show that the disease is transmitted by the corms.

It was therefore necessary to cultivate the corms under such circumstances that ordinary soil-fungi would be excluded. If the

corms then still became infected, it ought to be easier to isolate the pathogenic organism. As Calla-lilies under ordinary circumstances grow in marshes and swamps, it was probable that it would be possible to cultivate them in culture-solutions. As the big corms are awkward to work with I used very young ones in my experiments.

I took some young corms of infected plants, brushed half the number with water and the other half with a fairly strong solution of formalin (commercial solution diluted about 20 times). I then took a glass vesicle which had first been painted black and then white, so that the temperature of the liquid inside would not become too high. A lid, through which a few holes had been made, could be screwed on it. The vesicle was filled with culture solution of v. d. Crone:

1 L. water
1 gr. KNO_3
0,5 gr. $Ca\ SO_4$
0,5 gr. $Mg\ SO_4$
0,25 gr. $Fe_3\ (PO_4)_2$
0,25 gr. $Ca_3\ (PO_4)_2$.

The young corms were allowed to hang in the culture solution and were fastened in the holes in the lid by means of two pieces of cork, which fitted in each hole. In this way the leaves could freely develop into the air while the corms touched the culture-solution. After some time it became evident that the plants which had been treated with formalin, had developed excellently. The roots were strong and healthy. The roots of the plants which had only been treated with water, on the other hand, soon showed signs of infection. This rot could not be the result of an organism present in the solution, as the formalin-plants remained healthy.

When the infected roots of the plants in the solution were examined microscopically, they were found to be covered by a large number of sporangia, which soon formed swarmspores in water. Hardly any other fungi were present. When I layed out small pieces of these infected roots on agarplates the fungus only grew very slowly, although such a number of swarmsporangia were present.

I nevertheless succeeded in isolating it in this way as the usual soil-fungi were absent.

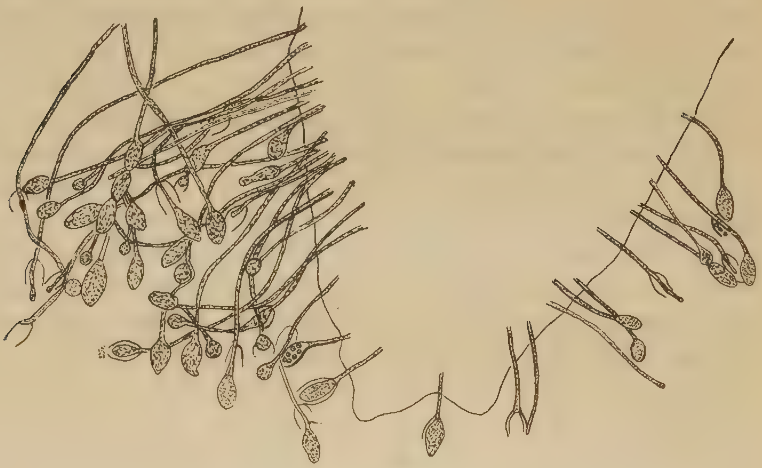

Fig. 4
Part of diseased Calla-root, crushed under the cover-slip.
± 80 ×

§ 3. The pathogenic organism

Inoculation experiments, which will be described below, proved, that the fungus with swarmsporangia which was found on the diseased roots was indeed the cause of the rot. I afterwards also found it on a root of a diseased plant which had not been grown in waterculture but had been taken out of the soil.

The way in which the swarmspores are formed could directly be followed when infected roots were examined in water. It appeared that this happens inside the sporangia in the way characteristic for Phytophthora.

It also became evident that, after the swarmspores have been set free, the sporangiophore grows into the sporangium in the same way as is found in the group of Phytophthora cryptogea, as I have already pointed out.

Little branched sporangiophores and sporangia without a distinct germinal papilla are also found.

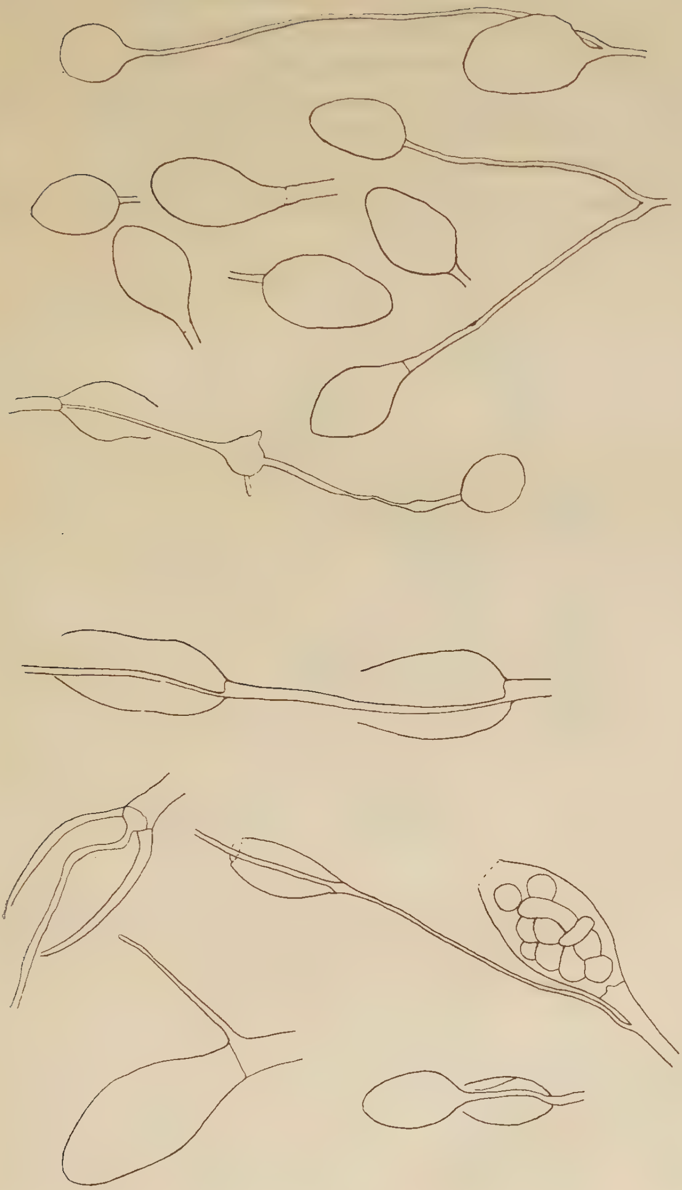

Fig. 5

Zoosporangia of Phytophthora Richardiae in culture-solution.

± 366 ×

Fig. 5

Many oogonia with amphigynous antheridia were present in cultures on oatmeal-agar, but sporangia never appeared. These were only produced when I applied the method described by Petri.

The Phytophthora of the Calla-lily is closely related to P. cryptogea, P. cambivora, P. gonapodyides and P. cinnamomi.

It differs from P. cinnamomi in the absence of chlamydospores. From the others it differs in the size of the oogonia. The oogonia of the Phytophthora of the Calla-lily generally measure ± 34 — 38 μ, while the average of those of P. cryptogea is given as 30 μ.

Fig. 6
Zoosporangia of Phytophthora Richardiae, grown on Calla root in v. d. Crone-solution. ± 400 ×

For the oospore of P. cambivora Petri gives measurements of 20—27 μ and for the oogonia of P. gonapodyides measurements of 22—36 μ are given by Miss Kanouse.

Besides, while the Calla-Phytophthora forms oogonia abundantly, P. gonapodyides and P. cambivora produce no oogonia in culture and P. cryptogea only a few on oatmeal-agar.

Oogonia are no longer produced by an old culture of P. cryptogea present in the collection of the Centraal Bureau voor Schimmelcultures. It was therefore impossible for me to compare the

oogonia of the Calla-Phytophthora with those of the three last mentioned Phytophthora's. Nevertheless I think, that the colour of the wall of the oogonium and oospore also differs from those described by Pethybridge for P. cryptogea.

In this way there are various characteristics in which the Phytophthora of the Calla differs from the forms closely related to it. Also the special occurrence in the roots of the Calla-lily and the virulent action on them, made me decide to form a new species *Phytophthora Richardiae*.

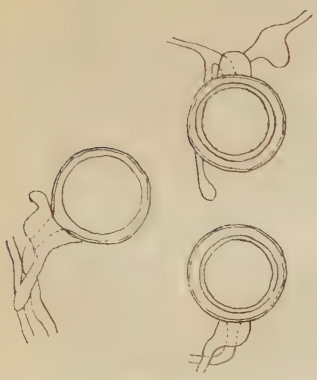

Fig. 7
Oogonia and antheridia of Phytophthora Richardiae. ± 366 ×

The description of this species is as follows:

The mycelium is the same as that of other Phycomycetes. Sporangia vary in size as is apparent in the drawings. They are produced by the method of Petri, or by cultivating plants in water-cultures and infecting the roots with the fungus. Zoospores reniform, biciliate, ± 10 μ in size, produced in varying numbers in one sporangium. Oogonia produced in oatmeal-agar and in tissues of infected roots, obovate, growing through antheridium, diameter of distal end ± 34—38 μ. Oogonial wall yellow. Oospores ± 29 μ in diameter, wall $2-2^1/_2$ μ. in diameter and greenish purple in colour.

Antheridia with protuberances, amphigynous, occurring on other hyphae than the oogonia.

Very virulent parasite of the roots of the Calla-lily causing them to decay. May also penetrate into the corms with the same effect.

§ 4. Inoculation experiments

It now had to be proved by inoculation experiments, that the Phytophthora is indeed the cause of the above mentioned disease. I first used small corms for these experiments because they are easier to work with.

The first inoculations were carried out on some of the young

corms treated with formalin. They were quite healthy and grew vigorously in the culture solution, although they had been taken from diseased plants. Small pieces of agar with Phytophthora mycelium were smeared on the roots. After a few days the roots began to show signs of decay, they became flabby and took on a glassy appearance.

This spread further and further until after some time all the roots were badly infected.

In order to be absolutely sure, I then inoculated corms of healthy plants which had also been brushed with formalin. These inoculations gave the same results as the previous ones. The outer leaf of an infected corm soon shows the same symptoms as appear in fullgrown diseased plants.

Finally I also infected large corms before planting them in pots, by rubbing a culture of Phytophthora on them. Corms treated in this way formed very few roots and on a whole showed the same symptoms of disease.

As the corms are sometimes only partly rotten, I wanted to find out if this was also due to infection by Phytophthora. I therefore laid slices of the corms in petridishes on wet filterpaper and inoculated them with the Phytophthora. A very peculiar rot, differing from the slimy bacterial rot, appeared. The cells seemed to become detached from each other and a fairly dry mass was formed.

§ 5. Control

It was now necessary to find a method of controlling the disease. As I had been successful in treating the corms with formalin it was probable that a solution to the problem could be found in this direction. Originally I had immersed the corms before planting them in a 2 $^0/_0$ solution of formalin and then brushed them with it.

As it appeared that this treatment was not sufficient, I think it better to leave them in a weaker solution for some time. The rotten places should be cut out.

Investigations of Bewley (5,6) already showed that the corms do not suffer by this treatment with formalin. Bewley reported an epidemic in Calla-lily nurseries, which seems to be very

Plate I

Two young corms of Calla after having grown for some time in v. d. Crone-culture-solution. They originated from a diseased plant, but were brushed with formalin before being placed in the culture-solution.

Plate 2

The two plants at the right originated from a diseased Calla and were handled in the same way as the plants in plate 1.

They were inoculated with Phytophthora Richardiae after the roots were well developed.

The young corm at the left originated from a healthy Calla and was also inoculated with Phytophthora Richardiae.

The roots have disintegrated into a glassy, afterwards slimy mass.

similar to the above described disease. He takes *Bacillus aroideae* Townsend to be the cause, but does not describe any inoculation experiments with this organism. Townsend (36) says about the symptoms of his disease: ,,The writer found the Calla's rotting off usually at or just below the surface of the ground, the disease sometimes extending down into the corm, sometimes upward into the leaves, and frequently in both directions. Occasionally the disease seemed to start in the edge of the leafstalk, in the flower stalk or in some underground part of the corm, though as a rule it started at the top of the corm just above, but near the surface of the ground".

These symptoms differ in many respects from the ones I noticed in the Calla-nurseries in Holland.

Although there seems to be a great similarity between the symptoms described by Bewley, and those of the disease in Holland, I am not able to say if the two diseases are identical, as I never had the chance of examining Bewley's specimens.

Bewley used a solution of formalin diluted from 1 to 49 for control measures. He leaves the corms in this solution for an hour, without any damage being done to them. This proved to be a very good way for controlling the English disease.

In the meanwhile, the formalin treatment has been applied at one of the Calla-lily nurseries in Holland, and it was found, that the disease was far less universal than before. It is hoped, that on applying the method a second time, the disease will be entirely got rid of.

CHAPTER IV

ROT OF CUTTINGS

§ 1. *General discussion*

Especially in the last few years many cases are reported of cuttings which became infected by different species of *Pythium*. In 1910 Peters (24) described the „Schwarzbeinigkeit" (Blackleg) of geranium caused by *P. de Baryanum*. In 1922 van Poeteren (30) also reports the presence of P. de Baryanum on geranium cuttings.

A publication on Blackleg of Pelargonium by W. Buddin and E. Wakefield (9) appeared in 1923, in which P. de Baryanum and Botrytis cinerea are named as the cause of the disease.

In 1924, H. Pape (23) reported P. de Baryanum as a parasite on carnation cuttings.

A detailed description of Pythium species occurring on geranium, was published by Braun (8) in 1925. He describes two new species, *Pythium splendens* and *P. complectens*.

In May 1925 some chrysanthemum cuttings, which had suddenly stopped growing, were sent to me to be examined. It became evident, that the roots were suffering from a rot. I soon succeeded in isolating a phycomycete from these decaying roots. This fungus grew well on oatmeal-agar; it produced no oogonia however, but a large number of conidia or swarmsporangia were formed in agar-cultures. In order to be able to identify it, it was necessary to find out in which way the zoospores were formed. I therefore placed

a tuft of the mycelium from agar-cultures in a watchglass with water. It appeared, that this is not the right method for obtaining zoospores. It was possible however to identify *this* fungus without seeing swarmspores, when it is placed in water.

If, for instance, the mycelium in the watchglass was allowed to stand for about twelve hours without the water being refreshed, numerous sporangia were formed in chains. The first formed specimen of such a chain often has a somewhat obovate shape, the others are round. This corresponds with the description of *Pythium intermedium* given by de Bary (4). He also mentions the absence of oogonia and antheridia. The chains of sporangia are so characteristic, that it is nearly impossible to mistake it for other Phycomycetes. De Bary found Pythium intermedium together with Pythium de Baryanum in dead seedlings of *Lepidium* and *Amaranthus*. Inoculation experiments however proved that it is impossible for P. intermedium to penetrate into these seedlings while they are living. But, according to de Bary it easily penetrates into the tissues of healthy prothallia *(Equisetum, Todea, Ceratopteris)*. P. intermedium is also mentioned at length by Butler in his publication on Pythium.

He states:

> „It is curious, that this species, which is common as a soil saprophyte, should have been so rarely observed. I have so regularly obtained it from water, in which were suspended fresh roots of *Abutilon*, that I at one time suspected parasitism on this plant. I however, failed to induce it to attack living roots. De Bary equally failed to infect Lepidium sativum and Amaranthus with it.
>
> He, however, observed its parasitism on prothalli. Atkinson found, that it was responsible for a disease of fern prothalli in the United States. The affected plants were soft, limp and darker in colour than healthy ones. A high temperature, moist soil and air, and insufficient light and ventilation, are the chief predisposing factors to damping-off. When the soil is infected, it should be replaced by fresh sterilized earth".

I also came across this species several times. From Louise Solberg (Oslo) I received a strain of the same fungus, which she

had isolated from diseased tubercles of a lupin. It is therefore probable, that P. intermedium often occurs in vegetable mould. It is easily distinguished from the other Phycomycete-types with round sporangia and without oogonia, which are so often found on decaying roots or in the soil, by the often long chains of sporangia, which are formed when the mycelium is placed in water.

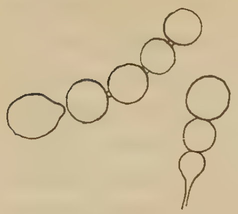

Fig. 8
Chains of sporangia or conidia of Pythium intermedium. 366 ×

The solution of Petri is again successful in obtaining zoospores.

If the mycelium is left for a few days in this solution and then transferred to a watchglass with pure water, swarmspores appear after a few hours or sometimes after a day. If the mycelium is straightaway transferred from agar-cultures to water most of the sporangia germinate as conidia.

Inoculation experiments now had to prove, that P. intermedium is indeed able to infect chrysanthemum-plants and cause a retardation in growth.

When I examined the infected plants two months afterwards to study the course of the disease, it appeared that it was not of a very serious nature. All plants, also those which had been most badly infected, had outgrown the disease. This was probably also due to more favourable conditions of temperature and humidity.

Up to now the presence of Pythium intermedium on chrysanthemum or other cuttings has not been reported. I also wanted to inoculate geranium cuttings with it, as they are apparently easily attacked by different Pythium species. It is naturally thought, that the fleshy, juicy stems of geraniums are more easily attacked by Phycomycetes than the woody stems of chrysanthemums.

§ 2. Inoculation experiments

For comparison I also carried out some inoculation experiments with *Pythium de Baryanum* isolated from peas, *Pythium complectens* and *P. splendens* obtained respectively from Braun and from Leonian and with a Phycomycete corresponding with *P. de Baryanum var. geranii* Braun.

This last fungus is one of those forms without oogonia which appeared when diseased roots were layed out on agarplates. It is characterized by fairly large, dark, round conidia and many chlamydospores of different sizes. I did not succeed in obtaining swarmspores or oogonia from it.

The best results with inoculation-experiments were obtained when the lower ends of the cuttings were rubbed with the mycelium from agar-cultures and then placed in pots with coarse sterilized sand. In order to get as much moisture as possible, the pots were placed in socalled ,,sun-catchers'', small glass houses built by means of thick iron wires.

After a few days, the first symptoms of the disease appear. The base of the cutting becomes black and the colour slowly spreads towards the top. This phenomenon was also discussed by Braun. The infection by Pythium splendens spreads through the whole cutting, while that of P. complectens stops a few c.M. from the base of the slip. I think that the typical dark black discoloration of the tissues of geranium is characteristic for infection by Phycomycetes, while with Botrytis-infection a more brown discoloration is typical. It appears, that on a whole geranium-cuttings are more easily infected than chrysanthemum cuttings.

It only seldom happens that a chrysanthemum cutting is so badly infected that it is killed at once. It is probable however, that the somewhat diseased plants may show a temporary retardation in growth.

As I always used fairly young slips I have not been able to determine if the rot may also attack the roots, as one would suspect when examining the originally diseased plants, which were much older than the cuttings used by me. In any case, very special conditions of temperature and humidity must appear, before such a disease will be the result.

Inoculation experiments proved that Pythium intermedium is strongly pathogenous for geranium cuttings, having the same effect on them as P. splendens. Chrysanthemum cuttings on the other hand, are only slightly attacked by it.

Pythium de Baryanum var. geranii, like P. de Baryanum proved to be pathogenic for chrysanthemum cuttings, while P. splendens also attacked chrysanthemum slips.

Some results of the inoculation experiments are given below:

1. Result of inoculation experiment in a hothouse with mean temperature about 20°, on 11th. February '26.

   6 geraniums and 1 chrysanthemum inoculated with P. splendens.

   On 16th. February: 5 geraniums and 1 chrysanthemum obviously infected, 1 geranium attacked at the base (this one died afterwards).

   6 geraniums and 2 chrysanthemums inoculated with P. complectens.

   These inoculations gave no result.

   6 geraniums and 11 chrysanthemums inoculated with P. intermedium.

   On 18th. February: 1 chrysanthemum and 4 geraniums obviously infected; 1 geranium black at the base, the others healthy.

2. Result of inoculation experiment carried out in the same hothouse on 23rd. April '26.

   12 cuttings of chrysanthemum and 6 cuttings of geranium inoculated with P. intermedium.

   On 26th. April: 1 chrysanthemum beginning to show black discoloration.

   On 29th. April: 4 geraniums turning black, 1 beginning to show discoloration.
   The other plants remain healthy.

3. Result of inoculation experiment carried out in the open in sterilized sand on 17th. and 18th. June 1926. (See table p. 27). As Pythium intermedium often occurs in vegetable mould, I presume, that blackleg of geranium is not only caused by Pythium de Baryanum, but also by P. intermedium. It is however necessary to isolate the fungus from the diseased geraniums in order to be able to say to what species the blackleg is due.

	Number of inoculated plants	Heavily affected	The infection stops on some distance from the base	Healthy
P. splendens	6 ger.; 6 chrys.	6 ger.; 1 chrys.	2 chrys.	3 chrys.
P. complectens	3 ger.; 3 chrys.		3 ger.	3 chrys.
P. intermedium	6 ger.; 6 chrys.	6 ger.	2 chrys.	4 chrys.
P. de Baryanum	6 ger.; 6 chrys.	4 ger.	2 ger.; 2 chrys.	4 chrys.
P. de B. var. geranii	6 ger.; 6 chrys.	5 ger.	1 ger.; 2 chrys.	4 chrys.
Control	5 ger.; 5 chrys.			5 ger.; 5 chrys.

CHAPTER V

ROOT ROT OF PEAS

§ 1. General discussion

In the last few years a great deal has been done in America towards investigating the root rot of peas caused by Phycomycetes. — Especially Fred. R. Jones did much in solving this problem. In 1923 (16) he drew attention to the fact, that a fungus which seemed to be a Phycomycete, is often found in the tissues outside the endodermis of the roots of Legumes and some other plants. He did not succeed in cultivating this organism outside the roots. When it was present in large quantities it seemed to cause a disease in the roots of Lathyrus, but under ordinary circumstances it did not appear to do much harm. Jones therefore regards it as a kind of mycorrhizal fungus.

Perhaps it is a Phycomycete, but nothing further has become known about it. F. R. Jones and his assistants however, afterwards investigated other Phycomycetes which occur specially on pearoots. A publication by Drechsler, *Root rot of Peas in the middle Atlantic States in 1924,* appeared in 1925. (11)

The Phycomycetes which he isolated from pearoots are:

1. 6—8 species of *Pythium* of which 3 are usually included in Pythium de Baryanum,
2. 2—3 species of *Artotrogus* (with thorny oogonia),
3. *Aphanomyces euteiches.*

   This last appeared to be the most pathogenic and is very fully described by F. R. Jones and Drechsler (17). The

disease especially appears on fields on which peas have been planted a few years in succession.

Drechsler further remarks about Pythium and Aphanomyces:

,,Although some isolations were made from discoloured rootlets not showing evidence of being attacked by any other fungus the most prolific source for cultures of species of Pythium was found in the cortical tissues of the stem and larger roots bearing the oogonia and oospores of Aphanomyces euteiches. In fields in which the latter fungus was common, the genus Pythium appeared to be found occurring more abundantly in secondary relationships than in directly parasitic ones. This condition is apparently not due to any lack of potential virulence, since nearly all of the smooth forms tried out so far, as well as one spiny form, which was derived from material collected at Hamburg, N. Y. attack cucumber fruits with great readiness, generally a fair index of a moderate degree of pathogenicity.

,,The widespread occurrence of even the most aggressively parasitic species of Pythium in dead organic matter has long been recognized, and it is evident that the tissues killed by Aphanomyces euteiches provide a more congenial substratum than the living parts".

F. R. Jones and Linford published an account called: *Pea disease survey in Wisconsin,* (18) in which the damage due to species of Pythium is treated in one chapter.

In this chapter he states:

,,When pea plants suffering from root rot are examined in the laboratory, the species of Pythium long known as a destructive seedling parasite will often be found present in the diseased tissue of many or all of the plants. Inoculation with some of the cultures of Pythium obtained in this way has shown, that the fungus is capable of preventing germination of pea seed or of destroying many of the seedlings before they emerge from the ground, and occasionally some degree of stem and root rot is produced.

,,The study of the relation of Pythium to root and stem rot has been greatly retarded by the fact, that the cultures obtained from peas in the field have been found to belong to several species differing somewhat in pathogenicity and frequency of occurrence and

furthermore it is not at all certain, that present methods used in making isolations from plants secure cultures of all species present. Thus a vast deal of work will be required before the relation of Pythium species to stem and root injury of mature plants will be fully known. It may be stated, however, that inoculations made under controlled conditions have shown very slight ability in any species studied thus far to produce either stem or root rot under usual field conditions.

,,During the summer, isolations were frequently made to secure cultures of any species of Pythium, that might be present. Since previous experience had shown, that some species are almost as frequently found in association with roots apparently healthy as with those diseased cultures were made from both. In making cultures no sterilizing agents were applied to the surface of decaying tissue because they penetrate rapidly and destroy all Phycomycetous mycelium quickly. Thus mycelium adhering to the outside of roots may give fise to a culture, a difficulty which can hardly be avoided.

The results of 76 isolations are summarized in a table:

	No culture of Pythium obtained	1 species of Pythium obtained	2 species of Pythium obtained
Healthy pea roots	10	22	14
Decaying pea roots and stems	6	16	8

,,This table corroborates previous experience that cultures of Pythium are obtained with approximately the same frequency from healthy as from diseased plants. It also shows that in about one-third of the isolations two species were obtained, though in such cases no two species seem to be found more frequently associated than others.

,,From the observations made thus far it appears that some of these species of Pythium occurring abundantly in the soil may at times be responsible for the death of root ends and for some rootlet injury, but only rarely for root and stemrot as it is usually known. It is possible that almost universal invasion of the root cortex of

peas and clover by a mycorrhizal fungus renders this tissue especially accessible to these species and that it is from this superficial invasion that the fungus is most frequently obtained in culture". —

In Europe, *Pythium Sadebeckianum* is reported occurring on peas. Butler (10), however, omits this species. He remarks about it: ,,This species occurred as a parasite on peas in Pomerania and lupines in Hamburg, doing a considerable amount of damage in 1891. The diseased peas were examined by Wittmack, who published a brief account of the pathological conditions without, however, any diagnosis of the fungus further than the measurements of bodies which resembled the oospores of a Pythium. Sadebeck, who saw the specimens considered it to be a new Pythium, allied to *P. de Baryanum* and *P. Equiseti.* The oogonia measured 32 μ in diameter. Whether it is the same as the species generally included under P. de Baryanum, which has been reported attacking peas in the United States, England and elsewhere is uncertain".

These are the most important publications which have appeared about the rootrot of peas caused by Phycomycetes.

In order to determine whether Aphanomyces euteiches is also found in this country, I examined several fields on which peas were cultivated. It was done in the first part of May as the plants are often attacked early in the season. Although a lot of rain fell in the spring of 1926 and conditions were therefore favourable for the development of the disease, I only found a few plants showing signs of disease. Only on one field a few rows of plants were apparently affected.

The affected plants first became yellow or mottled and then dried out. If the plants were taken out of the soil it could be observed that the roots showed signs of decay. I collected diseased plants from different parts and examined them microscopically. In the roots I found hyphae and sometimes oogonia belonging to some kind of Phycomycete, but I never met the peculiar hyphae of the mycorrhizal fungus described by Jones.

In isolating the fungus from diseased roots I of course had to cope with the same difficulties as Jones.

As the soil is full of Phycomycetes and the roots cannot be

sterilized, one never knows if an isolated species is actually the same one as that of which oogonia appear in the roots. Only inoculation experiments could prove if a certain isolated species would be pathogenic or not.

From most of the isolations a species was obtained with oogonia often possessing protuberances. I had met this fungus before in a young lupin which had shown the typical symptoms of damping-off.

When a colleague tried to isolate Cladosporium cucumerinum from cucumber seeds which failed to germinate the same species appeared in the isolations. Apparently it is a common soil fungus. Except this fungus with irregular oogonia, which I usually obtained from diseased roots, other forms appeared without oogonia and which were therefore hard to identify. I have however chiefly confined myself to the fungus with irregular oogonia which I shall afterwards describe as *Pythium irregulare n. sp.*

Besides I carried out some inoculation experiments on peas with one of the fungi without oogonia, which corresponds with P. de Baryanum var. geranii Braun. Although this fungus had not been isolated from peas it had appeared to be very virulent during experiments on cuttings. For comparison I also carried out some inoculation experiments with Pythium de Baryanum and Aphanomyces euteiches.

The former I had isolated from diseased pea roots, the latter was obtained as a pure culture from the United States (Drechsler).

§ 2. *Inoculation experiments*

For my first inoculation experiments I used humus, through which I mixed agarcultures of the fungi before sowing the peas in it. At the time I had not yet isolated Pythium irregulare from pea roots, so that I mainly used Pythium de Baryanum and P. de Baryanum var. geranii. It soon became evident that this method is not very satisfactory. The little radical was immediately attacked by the fungus and the peas did not come up. If they did, they either were very small and miserable or they outgrew the disease, but in neither case did they show the typical symptoms of root rot. Another drawback was that I had to use peas with a high percentage of germination, as it would otherwise be impos-

sible to carry out control experiments. ,,Wonder van Amerika", the variety I used, had a very high germinative power, but on the other hand it is more resistant to disease than other varieties of green peas (doperwten). When working with less virulent parasites there would therefore be more chance of the inoculation succeeding with less resistant varieties of green peas, than with the Wonder van Amerika. I arranged the inoculation experiments in the following way: the peas were sown in pots containing sterilized vegetable mould mixed with oatmeal-agar cultures of the different fungi in question.

The results of one of these experiments were:

	Number of seeds	Number of plants living after 11 days
Inoculated with:		
Pythium de Baryanum (isolated from pea-roots)	15	0
Pythium de Baryanum (strain from Hartley)	15	1
Pythium de Baryanum (Strain from diseased corm of Gloxinia)	6	1 poor specimen
Pythium de Baryanum var. geranii	15	3 (one of which very small)
Control	5	5

It therefore appeared that this method is not the right one. These difficulties could be removed by using waterсultures. One then also has a better survey of the progress of the rot. Other soil fungi which in pot experiments always follow on the Phycomycetes were eliminated in this way. The roots which have grown in waterсultures are clean and therefore very suitable for microscopical examination.

The seeds were allowed to germinate and the best seedlings were then sorted out and used for the waterculture-experiments.

For these experiments I used a green pea called Prins Albert.

It possesses long, thin roots, while seedlings of Wonder van Amerika have a short firm root system.

I proceeded as follows: The seeds were first sterilized with alcohol-sublimate (1 alcohol 96 % : 1 sublimate 0,1 % to prevent infection in the germinating dishes as far as possible.

They were then laid in dishes on wet filterpaper and when the roots were a few c.M. long they were used for the watercultures.

The watercultures were arranged as follows:

In the lid of a jampot three holes were made and in these holes two pieces of cork, somewhat hollowed out in the middle, were fitted. The roots of the seedlings were pushed through the round hole, which was formed between the two pieces of cork, while the cotyledons rested on the top of them.

The jampots were filled with the culture solution of v. d. Crone.

After the roots had grown in the solution for a little while, I placed a tuft of the mycelium from the fungus with which I wanted to inoculate, against them. It may of course be objected against this method that the fungus has an advantage over the plant in this way. A pea is not a waterplant and is therefore not cultivated under normal circumstances, when growing in watercultures.

Phycomycetes on the other hand are water-fungi and easily produce swarmspores in mineral solutions, so that in watercultures the circumstances are very favourable for infection. The pea seedlings, however, grow so well in v. d. Crone's culture solution, and produce such a healthy root system, that according to my opinion, they may well be compared with plants grown under normal circumstances. At the utmost one may get an exaggerated idea of the pathogenicity of the parasite, when one relies on the damage done by it to the root system of pea seedlings in watercultures.

But then the damage done to the green parts of the plant is usually less striking in watercultures than on the fields. On the fields the root rot is characterized by the drying out of the infected plants and this of course does not soon happen in watercultures: as long as a few roots remain intact the plant will not soon dry out. Although the whole aspect of the disease is therefore somewhat different in watercultures than in the pots, one may still draw conclusions about the pathogenicity of a special Phycomycete for the roots of peas. Aphanomyces euteiches appeared

to be the most virulent of the four Phycomycetes (Aphanomyces euteiches, Pythium de Baryanum, P. de Baryanum var. geranii and Pythium irregulare), with which inoculation experiments were done.

A little while after the pea roots had been inoculated with Aphanomyces euteiches the whole root system became limp.

They did not become as glassy and transparent as with Pythium inoculations but remained white. The infection is very acute. Even in watercultures the plants may shrivel up and die.

When inoculated with Pythium irregulare the roots were in many cases also soon badly infected. On the upper part of the plants nothing could be seen however. The growth was normal and the leaves were dark green. The roots were glassy, transparent and brown in colour, while there were only a few small side roots at the ends. These side roots may, when they are also infected, show a reddish brown ring at the place where they are attached to the main roots. When roots infected by Aphanomyces euteiches or Pythium irregulare are examined microscopically, large numbers of oogonia are found in them.

Pythium de Baryanum and P. de Baryanum var. geranii also attack pea roots in watercultures. In their method of infection they resemble Pythium irregulare and not Aphanomyces euteiches.

The plants in the control experiments remained healthy and possessed a clean white, well branched root system.

From these results I think I may conclude that Pythium irregulare is the cause of a root rot of peas, and that a large number of the diseased plants with decaying roots, which I found on the fields, is due to it. Finally it may be pointed out, that it is hard to distinguish Pythium irregulare from Pythium de Baryanum when the infected roots are examined microscopically.

The protuberances of the oogonia are very hard to find in the tissues of the roots.

In culture however they are easily distinguished.

§ 3. Description of Pythium irregulare nov. spec. in culture

Many of the isolations which I made of this Pythium showed different variations. I specially kept three of them in culture, one

from peas, one from lupins, one from cucumber seeds. Macroscopically these fungi look exactly like other Pythiums. I usually cultivated them on oatmeal-agar on which they made the usual white woolly growth of mycelium. I tried to get swarmspores by the method of Petri. The strain isolated from cucumber seeds produces a few swarmspores in this way, but there were too few to observe the discharge of the swarmspores from the sporangia, or to be able to count the cilia. For the purpose of counting the cilia, I placed a drop of water with swarmspores on a slide with a drop of eosine-sublimate (following the advice of Mr. v. Luyk).

The swarmspores were immediately fixated and coloured by the eosine before they withdrew the cilia. It is obvious that one can only apply this method when there are a fairly large number of swarmspores at one's disposal. It was impossible for me to count the cilia, as the strain from cucumber only produced a few, the strain from lupin only very few single ones, and the strain from pea no swarmspores at all.

As I could not determine the way in which the swarmspores were formed the difficulty was to decide whether the species belonged to Pythium or to Phytophthora.

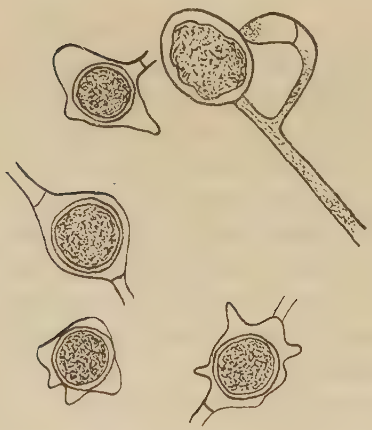

Fig. 9
Oogonia of Pythium irregulare isolated from cucumber-seed. 550 ×

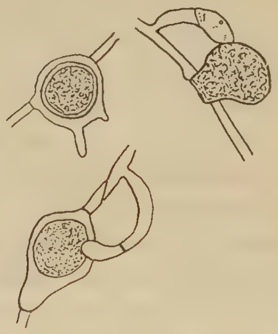

Fig. 10
Oogonia of P. irregulare isolated from pea-roots. 550 ×

I did not hesitate for one moment, however, in placing it with

the genus Pythium, owing to the great similarity which exists between this fungus and P. de Baryanum.

The only difference which really exists between this species and P. de Baryanum is the irregular shape of the oogonia. The oogonia often (but not always) carry protuberances, varying in size and

Fig. 11

1 Conidia of Pythium irregulare isolated from lupin. 550 ×
2 Empty sporangia and conidium of P. irregulare isolated from cucumber-seed. 550 ×
3 Germinating zoospores of P. irregulare isolated from cucumber-seed. 550 ×

shape, and sometimes separated from the oogonium by a transverse wall. These protuberances are especially found in cultures

a few weeks old. The oogonia are mostly intercalary and the oospores, sometimes two in number, lie free in the oogonium. The antheridia are usually of the same type as those of P. de Baryanum, but I never saw hypogynal antheridia. One oogonium is sometimes surrounded by more antheridia. The conidia or swarmsporangia are globose or somewhat obovate, and differ in size and shape.

I think that this fungus is sufficiently distinguished from P. de Baryanum by the protuberances found on the oogonia, to justify recording it as a new species for which I propose the name *Pythium irregulare*.

Pythium irregulare nov. sp.: Oogonia often with protuberances of different sizes and shapes.

Oogonia sometimes globose, sometimes irregular in shape. When globose, often $\pm$ 16—18 μ in diameter, usually intercalary. Oospore free in the oogonium, ordinarily $\pm$ 14—16 μ in diameter. The wall of the oospore has a thickness of about 1—1$^1/_2$ μ.

Antheridia produced on the same hyphae as the oogonium, sometimes several round one oogonium. Swarmsporangia and conidia vary greatly in size, $\pm$ 10—20 μ, globose or obovate. Swarmspores seldom produced, $\pm$ 8 μ in diameter when in rest. Isolated from pea roots, lupin plant and cucumber seeds. Distinguished from P. de Baryanum by the protuberances found on the oogonia.

CHAPTER VI

ROOT ROT OF FLAX

§ 1. General discussion

When examining pea roots microscopically I sometimes observed *Asterocystis radicis* de Wildeman (37) in the parenchymal cells. This fungus belongs to the Chytridiaceae and Marchal (21) described it as being the cause of the Belgian and Dutch *„vlasbrand"*, a name, which we might translate by „flax-fire". It is a typical wilt-disease, not identical however with the Fusarium flax wilt of the U. S.

In Holland the „vlasbrand" occurs principally in the northern provinces, Groningen and Friesland. When the disease is very acute large open spaces appear in the fields. The flax plants in these places are small, the leaflets wilt, they turn yellow and shrivel up. The plants look as if they were scorched by fire. Under favourable conditions the plants sometimes outgrow the disease, but they never quite recover.

The blue flax is much more susceptible than the white variety. In severe epidemics however, as in 1926, the white flax is also attacked. On old heavy clay soils, the „brand" rarely occurs, but on the lighter sandy clay, the disease is very common. It is remarkable that even in the new „polders", where flax is grown for the first time, the „brand" may occur. This disease is of a severe nature; the yield on those fields is extremely small. Dr. Zijlstra, chief of the botanical department of the Groningen Experiment Station, called it „a pest for our best flax regions".

Marchal found Asterocystis radicis in all the ,,brand" specimens from different parts of Belgium. He proved the parasitic nature of this fungus by carrying out inoculation experiments. He grew flax in watercultures and inoculated the nutrient solution with Asterocystis; the result was a thorough infection of the roots. By growing the plants in infected soil, he also obtained diseased plants. The presence of Asterocystis in the roots is easily ascertained by a simple staining process with potassium-iodide. Marchal then determined that the chlamydospores and swarm-sporangia were stained a deep brown-red. Where the symptoms of the disease were concerned Marchal remarks: ,,The less water the plants have at their disposal, the stronger they react on the lack of water, which becomes evident after infection and the more striking are the symptoms of the ,,brand" ".

The moisture, which by influencing the dissemination of the swarmspores helps to spread the disease, on the other hand diminishes its pernicious influence.

Marchal's investigations are the only ones published about this disease. Dr. Zijlstra informed me how to obtain infected plants in spring. He therefore sent me a sample from ,,brand" soil, which had been collected two and a half years ago. He advised me to grow flax in nutrient solutions (van der Crone). The bottom of the jar was covered with a thin layer of the soil. This infection succeeded beautifully. A large part of the root-cells was filled up with the Asterocystis sporangia. The plants of course, having their roots in the liquid, did not shrivel like those in the field, although they had a sickly appearance. The control plants were quite healthy.

When growing peas in the same solution, I also noticed a slight infection of the roots. In this case however the plants remained quite healthy.

In the summer of 1926 I visited the flax district of Groningen and collected plants of a ,,brand" spot in the field. I was there told by one of the foremost farmers, that these plants were typical ,,brand" plants.

The examination of these specimens however revealed relatively few sporangia of Asterocystis, but a great many round spiny bodies, which struck me as being oogonia of a Phycomycete. Later

in the summer I had the opportunity of examining flax roots from Friesland, and again I noticed the same phenomenon; the spores of Asterocystis however were more abundant in the roots here.

The question now arose, whether the root rot of flax might be caused by the Phycomycete in question, as well as by Asterocystis radicis. It would not be surprising if the wilting and shriveling of the plants were caused by different parasites. The ,,vlasbrand'' would then be a complex of diseases.

It is possible that several kinds of root parasites might nearly have the same effect. We know f. i. that Thielavia basicola may cause a rootrot of flax. —

Fig. 12
Part of flax-root crushed under the cover-slip after inoculation with P. megalacanthum. ± 70 ×

The Phycomycete proved to be *Pythium megalacanthum* de Bary (4), which was isolated very easily from the decaying roots. I thus intended to make inoculation experiments on the roots, with the exclusion of Asterocystis. For this purpose the flax seeds were germinated on wet filter-paper. The young plants were put in the nutrient solution and mycelium from the pure cultures on oatmeal-agar was smeared on the rootlets.

During the first days after the inoculation the roots apparently develop normally. After some days however, the top of the principal root begins to soften. This ,,rotten'' end is separated from the healthy part by a red ring.

As the rotting process advances, the ring is slowly replaced.

Except the main root, the lateral roots are also attacked. All these infected roots become glassy and limp.

Fig. 12 shows a piece of flax root from such an artificially infected waterculture. The spiny oogonia appear in large numbers in the roots.

It therefore appears that it is possible to cause a rot of the flax roots by Pythium megalacanthum, even when Asterocystis radicis is absent.

As Marchal already noticed with his experiments with Asterocystis, the plants in waterculture do not suffer very much from wilting. This also counts for Pythium megalacanthum. The aerial parts of the plants do not shrivel up.

I should have done experiments in pots, but I could not find the opportunity.

It was only my intention to focus the interest on the fact that „vlasbrand" is not such a simple disease, as one would like to imagine.

Other fungi may also be responsible for this root rot.

Before methods of control may be found, it will be necessary to analyse the different factors of this complex-phenomenon.

§ 2. Pythium megalacanthum de Bary

The large spiny oogonia of the Phycomycete which I found in flax roots, naturally reminded me of the illustrations of the oogonia of *Pythium megalacanthum* given by de Bary. De Bary found Pythium megalacanthum in seedlings of garden-cress which had already been attacked by Pythium de Baryanum. He tried to infect the seedlings by inoculating them with Pythium megalacanthum but did not succeed. He then inoculated prothallia of the fern Todea africana with it and some of them were attacked. He always used zoospores for his inoculations. According to Butler this fungus has never been described again except on one occasion when Schröter states, that he believed he found it in the stems of Veronica hederaefolia.

The Phycomycete which I isolated from flax seemed to correspond very well with the description given by de Bary for P. megalacanthum. The oogonia were often larger in size than the

measurements given by de Bary, but they vary so much that this is of no importance. De Bary already pointed out that the spores of P. megalacanthum vary more in size than those of P. intermedium. The drawing of the infected flax root (fig. 12) shows the variations in size of the oogonia, which range from ± 30 to 70 μ. I did not succeed in getting zoosporangia. Amongst his different cultures on seedlings of garden-cress, de Bary also had one form which possessed numerous oogonia and only few swarmsporangia. Unfortunately I only possess one isolation and perhaps that is the reason for the absence of the swarmsporangia. Even the method of Petri gave no results. P. megalacanthum grows on oatmeal- and on cornmeal-agar but not on cherry-agar.

The cultures have a granular appearance owing to the many large oogonia. Many oogonia are quite empty and the contents have degenerated. This was also noticed by de Bary who takes them for unfertilized oogonia.

Sometimes a few antheridia are present round one oogonium.

CHAPTER VII

MYCOLOGICAL ANALYSIS OF THE VEGETABLE MOULD

In connection with the inoculation experiments, it was necessary to know whether many Phycomycetes appear in vegetable mould in which the various diseased plants, which I had examined, grew. If many Phycomycetes occurred, the experiments with unsterilized soil would be worthless. With my first experiment in which the peas were sown in unsterilized soil, I already noticed that an infection by Pythium de Baryanum appeared in the control experiment. Unsterilized soil could therefore not be used.

When a decaying root is laid in a petridish on cherry- or on oatmeal-agar several Pythiums often start growing out of it. In the chapter about the root rot of peas I already pointed out that according to Drechsler (11) this may happen even if the rot is caused by a different parasite (in that case by Aphanomyces euteiches). F. R. Jones and Linford (18) even isolated several Pythiums from *healthy* pea roots.

One may gain an impression of the great number of Phycomycetes occurring in vegetable mould by laying out roots of different plants.

The many types which appear, make a research about Phycomycetes rather difficult at first.

One comes across several forms without oogonia or without swarmsporangia. With such isolations one often meets Pythium de Baryanum and the form called P. de Baryanum var. geranii by Braun. These forms were also often isolated from various seedlings.

One of my colleagues tried to isolate Cladosporium cucumer-

inum from cucumber seeds which failed to germinate in vegetable mould, but on one occasion he obtained different Pythiums instead. He handed them over to me and it appeared that Pythium intermedium and Pythium irregulare were present. From these seeds I also isolated a form with dark, somewhat spiny oogonia. I afterwards never came across this form again. Although the seeds were put to germinate in a hothouse, the temperature could hardly be the cause of the occurrence of this form, as many of my own seedlings were also cultivated at about the same temperature.

From decaying roots of pansies which had been grown in another soil-sample at a lower temperature, I isolated an *Aphanomyces* which looked very similar to Aphanomyces euteiches, but which, in my infection experiments, did not attack pea roots. Unluckily I did not have time to carry out inoculation experiments with it on pansies, and I have therefore not been able to ascertain whether this Aphanomyces is the cause of the acute root rot of pansies appearing in our experimental garden. From Calla-lily-roots I isolated, besides Phytophthora Richardiae, a few other forms,probably also Pythiums.

I never obtained Phytophthora species in the same way and apparently they do not lead the same saprophytic life in the soil as the different species of Pythium. The question of the temperature, which may be of importance here, was not investigated however. —

It is evident that vegetable mould is full of different Phycomycetes and that inoculation experiments with unsterilized humus would not be trustworthy.

It would lead me too far to discuss the different forms in this publication.

I also made some isolations from clay-soil. The difficulty with these isolations was that the dishes were often overgrown by a Mucor. But here again other forms than those which I had isolated from vegetable mould appeared.

SUMMARY

1. It has become evident that there are no sound reasons for regarding *Pythiomorpha* Petersen and *Blepharospora* Petri as constituting new genera. There is no objection against including them in the genus *Phytophthora*.

In practice it is not desirable that the genera Pythium and Phytophthora should be placed in one genus, although previous investigators have shown that theoretically there is no difference between them.

2. Inoculation experiments proved that a serious root rot of Calla-lilies, which causes a considerable damage in the nurseries, is caused by a Phytophthora species. As this species is not identical with any of the ones previously described, the name *Phytophthora Richardiae* n. sp. is proposed.

A treatment of the Calla corms with formalin, which has already yielded good results in practice, is given as a control measure.

3. It appeared that *Pythium intermedium* de Bary, isolated from chrysanthemum cuttings, which had stopped growing, is strongly pathogenic for geranium cuttings and may also attack chrysanthemum cuttings. Inoculation experiments were also undertaken with geranium cuttings, as a publication by Braun about the rot of these slips appeared in the United States.

For comparison I also inoculated with the species *P. splendens* and *P. complectens* named by Braun. These species, which had been kept in culture at the Centraal Bureau voor Schimmelcultures for some time, were still very virulent. P. splendens also attacked chrysanthemum cuttings. Inocula-

tion experiments with the strains of P. de Baryanum and P. de Baryanum var. geranii which I isolated, gave positive results with geranium and chrysanthemum cuttings.

4. A Pythium with irregular oogonia sometimes with protuberances, was isolated from the roots of a large number of the pea plants I found suffering from root rot.

As far as I know this species has not been described before and I therefore give the description and propose the name *Pythium irregulare* n. sp.

Inoculation experiments in watercultures proved that it attacks the roots of peas and is the cause of a root rot. I never came acrose Aphanomyces euteiches in peas, but inoculation experiments showed that it is more virulent than Pythium irregulare.

5. Typical *„vlasbrand"* has been ascribed to Asterocystis radicis de Wildeman.

The symptoms of „vlasbrand" of course occur whenever something is the matter with the roots. When examining the diseased plants I often found *Pythium megalacanthum* de Bary present in the roots. Inoculation experiments showed that this species attacks the roots of flax. As I found this species in the roots of diseased plants from different districts I think I may conclude that like Asterocystis radicis it may be one of the causes of the disease. A thorough re-investigation of vlasbrand is however necessary.

6. Inoculation experiments with Phycomycetes on roots are very much simplified by using watercultures.

7. It appeared that various Phycomycetes may be isolated from decaying parts of plants grown in vegetable mould. These forms are often very hard to identify owing to absence of oogonia or sporangia.

CITED LITERATURE

1. S. F. ASHBY, Oospores in cultures of Phytophthora faberi.
   1922, Kew Bull. Misc. Inf. 9, p. 257.
   Abstract in The Review of Applied Mycology II p. 183.
2. J. T. BARRETT, Pythiacystis related to Phytophthora.
   1917, Phytopathology 7, p. 150.
3. A. DE BARY, Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze.
   1879, Abh. Senck. Ges. XII, p. 225.
4. A. DE BARY, Zur Kenntniss der Peronosporeen.
   1881, Bot. Zeitung, 39 Jahrgang nrs. 33—39.
5. W. F. BEWLEY, Soft rot of the Arum.
   1921, Seventh Annual Report, Exp. and Research Station, Turner's Hill, Cheshunt.
6. W. F. BEWLEY, Soft rot of the Arum.
   1924, Tenth Annual Report, Exp. and Research Station, Turner's Hill, Cheshunt.
7. H. BRAUN, Geranium stemrot caused by Pythium complectens.
   1924, Journ. Agr. Res. Vol. 29, p. 399.
8. H. BRAUN, Comparative studies of Pythium debaryanum and two related species from geranium.
   1925, Journ. Agr. Res. Vol. 30, p. 1043.
9. W. BUDDIN and ELSIE WAKEFIELD, Blackleg of Pelargonium cuttings.
   1924, Gard. Chron. 75.
   Abstract in The Rev. of Appl. Mycology III, p. 455.

10. E. J. BUTLER, An account of the genus Pythium and some Chytridiaceae.
1907, Mem. Dep. of Agric. in India, Botan. Series I, nr. 5.

11. CHARLES DRECHSLER, Root rot of peas in the middle Atlantic States in 1924.
1925, Phytopathology 15, p. 110.

12. CHARLES DRECHSLER, Pythium infection of cabbage heads.
1925, Phytopathology 15, p. 482.

13. J. DUFRÉNOY, La vie parasitaire et la vie saprophytique des Phytophthorées.
1926, Revue générale des Sciences, mars 15.

14. H. M. FITZPATRICK, Generic concepts in the Pythiaceae and Blastocladiaceae.
1923, Mycologia 15, p. 166.

15. C. H. GADD, Phytophthora faberi Maubl.
1924, Annals of the Royal Botanic Gardens, Peradeniya, Vol. IX. Part. 1, June.

16. F. R. JONES, Mycorrhizal fungi in the Roots of Legumes and some other Plants.
1924, Journ. Agr. Res. Vol. 29, p. 459.

17. F. R. JONES and C. DRECHSLER, Root rot of Peas in the United States caused by Aphanomyces euteiches n. sp.
1925, Journ. Agr. Res. Vol. 30, pag. 293.

18. F. R. JONES and M. B. LINFORD, Pea Disease Survey in Wisconsin.
1925, Agr. Exp. Station of the Univ. of Wisconsin, Madison, Research Bulletin 64.

19. BESSIE KANOUSE, Physiology and Morphology of Pythiomorpha gonapodioides.
1925, Bot. Gazette Vol. 79, nr. 2, p. 196.

20. LEON H. LEONIAN, Physiological Studies on the genus Phytophthora.
1925, Amer. Journ of Bot., Vol. 12, No. 7.

21. EM. MARCHAL, Recherches biologiques sur une Chytridinée parasite du lin.
1900, Gembloux, Oct.

22. M. VON MINDEN, Beiträge zur Biologie und Systematik einheimischer submerser Phycomyceten.
1916, Mykol. Unters. und Berichte von Dr. R. Falck, II, p. 146.

23. H. PAPE, Ueber eine durch Pythium de Baryanum Hesse verursachte Stecklingskrankheit der Nelken.
1925, Die kranke Pflanze II, 4. p. 64.
Abstract in The Rev. of Appl. Mycol. IV p. 544.

24. L. PETERS, Eine häufige Stecklingskrankheit der Pelargonien.
1910, Zeitschr. für Garten- und Blumenkunde, 59 Jahrgang.

25. HENNING E. PETERSEN, An account of Danish Freshwater-Phycomycetes with biological and systematical remarks.
1910, Annales Mycologici VIII.

26. GEO. H. PETHYBRIDGE and H. A. LAFFERTY, A disease of tomato and other plants caused by a new species of Phytophthora.
1919, Scient. Proc. Royal Dublin Society, Vol. 15, Nr. 35.

27. L. PETRI, Studi sulla malattia del Castagno detta „dell' inchiostro".
1918, Annali del R. Ist. sup. forest. naz. III.

28. L. PETRI, Ulteriori ricerche sopra la Blepharospora cambivora.
1922, Ann. del. R. Ist. sup. forest naz. VII.

29. L. PETRI, Nuove osservazioni sulla biologia e sul parassitismo della Blepharospora cambivora.
1924, Ann. del. R. Ist. sup. forest. naz. IX.

30. N. v. POETEREN, Verslag over de Werkzaamheden van den Plantenziektenkundigen Dienst in de jaren 1920 en 1921.
1922, Juli, Wageningen.

31. R. D. RANDS, Streepkanker van kaneel, veroorzaakt door Phytophthora Cinnamomi n. sp.
1922 Mededeelingen van het Instituut voor Plantenziekten, Batavia, Nr. 54.

32. J. ROSENBAUM, Studies of the Genus Phytophthora,
1917, Journ. Agric. Res. Vol. 8, p. 233.

33. L. SOLBERG, Sygdom paa Erter.
1925, Havedyrkningens Venners Medlemsskrift, 4. hefte.

34. L. SOLBERG, Syke stedmorsblomster.
1926, Havedyrkningens Venners Medlemsskrift, 2. hefte.

35. R. E. SMITH and E. H. SMITH, Further studies of Pythiaceous infection of deciduous fruit trees in California.
1925, Phytopathology 15, nr. 7, p. 389.

36. C. O. TOWNSEND, A soft rot of the Calla-lily.
1904, U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin no. 60.

37. E. De WILDEMAN, Asterocystis radicis n. sp.
1893, Notes Mycologiques. I.
Extrait des Ann. de la Société belge de microscopie (Mémoires) XVII.

ERRATUM

Page 9, 24, 25, 27, 32, 33, 35, 44, 47, read *Pythium de Baryanum var. pelargonii* Braun instead of *Pythium de Baryanum var. geranii* Braun.

BRUNCHORSTIA DESTRUENS ERIKSS. AUF PINUS LARICIO VAR. CORSICANA UND IHRE REINKULTUR

VON

A. VAN LUIJK

In einer Anpflanzung von Pinus laricio var. Corsicana in einem Revier der „Maatschappij van Weldadigheid" zu Frederiksoord (Holland) trat im Jahre 1926 ein Zweigsterben auf. Der Direktor der forstlichen Versuchsanstalt, Herr Dr. E. Hesselink, machte uns darauf aufmerksam. Er meinte hier mit einem Befall durch Brunchorstia destruens zu tun zu haben. Eine Untersuchung an Ort und Stelle zeigte uns, dass ein ziemlich grosser Bestand von der Krankheit befallen war; eine Anzahl Bäume im Zentrum waren schon ganz abgestorben, andere sehr krank, während an der Peripherie des Krankheitsherdes alle Stadien der Krankheit auftraten. Weder an den Wurzeln noch an den Stämmen der kranken Bäume waren direkte Beschädigungen zu erkennen, ausgenommen an einzelnen Stücken, welche bisweilen Krebsknoten zeigten, eine Krankheitserscheinung, welche auch sonst bei der Corsikanischen Kiefer vorkommt. Es war klar, dass im allgemeinen der Tod der Bäume eine Folge des Zweigsterbens war.

Die Krankheit äussert sich zuerst dadurch, dass die Nadeln der befallenen Zweigen eine rostbraune Farbe annehmen. Eine typische Erscheinung bei dieser Krankheit ist, dass die Verfärbung und das Absterben an der Basis der Nadeln anfängt, während bei anderen Krankheiten und Beschädigungen die Erscheinungen sich zuerst an der Spitze oder in der Mitte der Nadeln offenbaren. Endknospen sterben bald ab; es ist sehr wahrscheinlich, dass die

Infektion an den Knospen stattfindet, wie von den meisten Forschern angenommen wird. Der Pilz dringt dann von da aus in den Zweigen und Nadeln.

Die von uns wahrgenommenen Krankheitserscheinungen stimmen ganz überein mit denjenigen, welche zuerst von Brunchorst (1) im Jahre 1887 für Pinus laricio austriaca in Norwegen beschrieben worden sind. Eine ähnliche Krankheit von Pinus silvestris ist in Preussen im Jahre 1895 von Schwarz (4) studiert worden, später wird das Auftreten der Krankheit auf verschiedenen Pinusarten noch aus N. Amerika von Weir (6), aus Norwegen von Jorstad (3), und aus Schottland und England von Waldie (5) berichtet.

Als Ursache der Krankheit wird von Brunchorst ein von ihm beschriebener, jedoch nicht benannter Pilz angegeben, der zu den Sphaeropsidales gehört. Eriksson hat diesem Pilze den Namen Brunchorstia destruens gegeben.

Derselbe hat ein- oder mehrkammerige, meist unregelmässig aufreissende Fruchtkörper mit spindelförmigen, gekrümmten Konidien ,welche in reifem Zustande 3—4 Querwände besitzen und 22—50 μ lang und 3—4 μ dick sind.

Wir haben auf den befallenen Zweigen in den verschiedenen Stadien der Krankheit nur wenige Fruchtkörper von Brunchorstia destruens gefunden, dagegen wohl öfters mehrere andere Pilze, von denen Zythia pinastri am zahlreichsten vertreten war. Bei unseren Isolierungen von der Grenze der kranken und gesunden Gewebe der befallenen Zweigen bekommen wir auch oft diesen Pilz, jedoch daneben auch oft ein in der Kultur meist grünlicher Pilz, der anfangs steril blieb, jedoch später fruktifizierte und sowohl in seiner Fruktifikation wie in seinem Mycelwachstum vollkommen mit der Brunchorstia destruens übereinstimmte, welche wir direkt aus Konidien isoliert hatten.

Ueber die Eigenschaften dieses Pilzes in der Kultur finden wir nur bei Waldie (5) etwas erwähnt. Er sagt, dass einzelne Sporen in einem Tropfen Wasser in 36 Stunden keimen. Der Pilz wächst bei ihm sehr leicht auf Agarboden mit verschiedenen Extrakten und bildet ein weisses, sammetartiges, oberflächliches Mycel. Fruktifikation wurde nicht beobachtet.

Wir haben den Pilz auf verschiedenen Nährböden gezüchtet und erhielten folgende Resultate: auf synthetischen Nährböden

wie Burgeff B, Czapek, Mannit-Agar, entwickelt der Pilz sich sehr schlecht, nur auf dem Impfstück und mitunter ein paar m.m. ringsum entwickelt sich eine sammetartige Myceldecke, welche bald weiss, bald gelblich und wohl auch grün gefärbt ist. Der Pilz dringt auch in den Agarboden hinein und bildet dort ein ziemlich dichtes stromaartiges Gewebe, welches bräunlich grun bis braunschwarz gefärbt ist.

Am meisten typisch ist der Pilz auf Maismehl. Er durchwächst den ganzen Nährboden und ist lebhaft grün gefärbt in allen Farbennuancen von gelbgrün bis grünschwarz. (Klincksiek et Valette, Code des couleurs, Nrs 251, 276, 301 en 310). Fruktifikation haben wir auf diesem Nährboden nicht wahrgenommen. Auf sterilen Koniferenzweigen bildet der Pilz ein grünliches Luftmycel, hier bilden sich auch die Pykniden. Diese zeigen sich gleichfalls auf verschiedenen Stengelkulturen (Lupinen, Tomaten, Kartoffel), doch ist hier das Luftmycel weiss.

Auf verschiedenen Agar-Nährböden mit Stärke (Hafermalz, Kartoffel u.s.w.) wächst Brunchorstia viel besser als auf synthetischen Böden, hat jedoch auch in diesem Falle noch ein gehemmtes Wachstum. In einer 3 Monaten alten Kultur auf Kartoffelagar z.B. ist nur ein Drittel der Agar-Oberfläche von dem Pilze bewachsen. Auf Kartoffelagar ist die sammetartige Luftmycelschicht graugrün gefärbt, auf anderen Stärkeböden ist das Luftmycel viel kräftiger entwickelt und weiss, jedoch ist das in den Agar eingesunkene stromaartige Mycel hier gleichfalls grün bis schwarzgrün gefärbt. Direkte Fruktifikation ist auf einer Mischung von Dekokten: Hafermalz und Pflaumendekokt, unter Zufügung von ½ % Pepton. Hier ist der Nährböden fast ganz bewachsen und zeigen sich hier und da die Stromata von Brunchorstia. Eine Eigentümlichkeit von Brunchorstia ist, dass oft der Nährböden an der Rückseite vom Glaswand des Röhrchens loslässt und der Pilz dann gerade an diesen Stellen fruktifiziert. Man kann diese Art des Fruktifizierens hervorrufen in Petrischalen, indem man von einer einige Wochen alten Kultur die Agarschicht umdreht; nach einigen Wochen brechen dann einzelne Fruchtkörper aus der überdeckenden Agarschicht hervor.

Die beste Entwicklung zeigt Brunchorstia auf einer Mischung von Kirschendekokt und Dünger-Agar, auch Kirsch-Agar und

Bierwürze-Agar und Gelatine sind geeignete Nährböden. Der Pilz überwächst hier die ganze Nährbödenoberfläche, bildet meist wenig Luftmycel und mehr oder weniger zahlreiche Früchtkörper.

Die in der Kultur entstandenen Früchtkörper sind wie die auf den Zweigen gebildeten ein- oder mehrkammerig, bisweilen entstehen ziemlich grosse zusammengestellte Stromata mit zahlreichen Kammern. In reifem Zustande entstehen an den Früchtkörpern runde oder unregelmässige Poren aus welchen die Konidien als gelbliche bis schmutzigrosafarbene Prfopfen zum Vorschein treten. Ausser den für diesen Pilz bekannten spindelförmigen, gekrümmten, septierten, grossen Konidien, welche ganz mit den Literaturangaben übereinstimmen, fanden wir in vielen Stromata in denselben Hohlräumen auch noch Mikrokonidien. Diese sind stäbchenförmig, gerade oder ein wenig gebogen, 4—5 μ lang und 1,25—1,75 μ breit. Sie werden endständig oder seitenständig gebildet auf mehr oder weniger stark verzweigten Konidienträgern. Ueber das Vorkommen dieser Mikrokonidien bei Brunchorstia fanden wir in der Literatur nichts erwähnt.

Schwarz (4) betrachtete Cenangium abietis als Ursache dieser Krankheit und Brunchorstia als eine der Konidienformen, während schon früher von Tulasne und Fuckel Dothichiza ferruginosa mit einzelligen Konidien gleichfalls als Nebenfruchtform von Cenangium abietis angesehen wurde. Auch Schwarz hält die Form mit einzelligen Konidien für eine Nebenfruchtform von Cenangium. Spätere Autoren sind in dieser Hinsicht weniger sicher. Diese Meinungen beruhen auch nur auf das in der Natur Beisammenfinden der verschiedenen Formen. Gewissheit gewinnt man selbstverständlich nur wenn man die verschiedenen Formen in Reinkultur züchtet.

Wir waren noch nicht in der Gelegenheit Infektionsversuche anstellen zu können, doch hoffen wir hierüber später Mitteilungen zu machen.

LITERATUR

1. BRUNCHORST, J. Ueber eine neue, verheerende Krankheit der Schwarzföhre (Pinus austriaca Höss).
   1888, Bergens museums aarsberetnung 1887. Nr. VI.
2. BRUCE, FINK. Injury to Pinus Strobus caused by Cenangium abietis.
   1911, Phytopathology I, p. 180.
3. JORSTAD, I. Norske Skogsygdommer, p. 49.
   1925.
4. SCHWARZ, F. Die Erkrankung der Kiefern durch Cenangium abietis.
   1895, Jena.
5. WALDIE, JOHN S. L. Die-Back Disease of Pines.
   1926, Transactions of the royal scottish arboricultural Society. Vol. 40, Part. 2, p. 120.
6. WEIR, J. Note on Cenangium abietis (Pers.) Rehm on Pinus ponderosa Laws.
   1921, Phytopathology 11, p. 166.

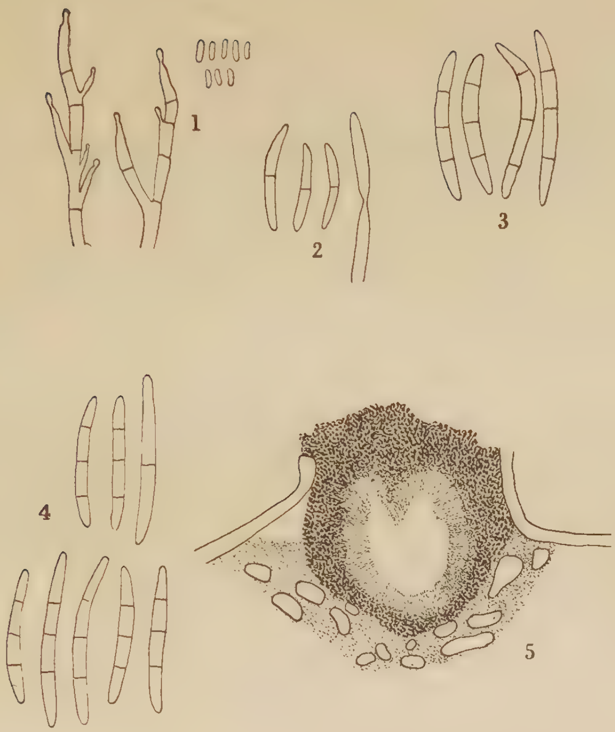

Fig. 1. Konidienträger mit Microkonidien van Brunchorstia destruens in Reinkultur. 750 × vergr.

Fig. 2. Konidienträger und junge Macrokonidien von Brunchorstia destruens im selben Fruchtkörper als Fig. 1. 750 × vergr.

Fig. 3. Reife Macrokonidien in Kultur auf sterilem Kartoffelstengel. 750 × vergr.

Fig. 4. Macrokonidien aus Fruchtkörper von einem toten Zweig von Pinus laricio corsicana. 750 × vergr.

Fig. 5. Fruchtkörper von Brunchorstia destruens auf totem Zweig von Pinus laricio. 50 × vergr.

PILZKULTUREN IN FEUCHTEN KAMMERN

VON

A. VAN LUIJK

Zum Zuchten von Pilzen werden als feuchten Kammern meist auf Objektgläsern befestigten Glasringe benutzt, welche von oben mit einem mittels Vaselin auf dem Ring aufgeklebten Deckgläschen abgeschlossen werden. Der Pilz wird dann im hängenden Tropfen an der Unterseite des Deckgläschens gezüchtet und kann auf diese Weise bei starker Vergrösserung betrachtet werden.

Ein Nachteil dieser Methode ist dass die Pilze sehr wenig Luft zur Verfügung haben, da die feuchte Kammer, um das Austrocknen zu verhindern, luftdicht abgeschlossen sein muss. Wir haben mit mehreren Pilzen die Erfahrung gemacht, dass die Sporen in diesem kleinen Raum nicht keimen, während in grösseren feuchten Räumen wohl Keimung stattfindet. Dasselbe ist u.A. mit einigen Ustilagineen der Fall. Für schnellwachsende Pilze ist der Raum manchmal auch zu beschränkt.

Eine Methode wobei diese Nachteile nicht auftreten ist diejenige welche Neger für die Züchtung von Russtaupilzen angewendet hat. Hierbei werden nämlich auf die Unterseite des Deckels einer gewöhnliche Petrischale mehrere Tropfen des Nährbödens angebracht, während eine Schicht Wasser auf dem Boden die Feuchtigkeit des Nährbödens konstant erhält. Der grösste Nachteil dieser Methode ist aber dass durch die Dicke der oberen Schalenhälfte die Pilze nur bei schwachen Vergrösserung wahrgenommen werden können.

Um nun die Vorteile beider Methoden vereinigen zu können,

haben wir folgende Anordnung ersonnen. In der oberen Schalenhälfte einer Petrischale wird ein runder Loch von 3,5 c.M. Durchmesser gemacht. Um den Rand dieser Offnung herum werden einige Tropfen geschmolzene Gelatine angebracht, darauf ein Deckglas von ausreichender Grösse (wir verwenden 40 $\times$ 50 m.m.) erwärmt und oben auf den Deckel gelegt sodass das Loch durch das Deckglas mittels der dünnen Schicht Gelatine vollkommen geschlossen ist. Um sicher zu gehen und eine eventuelles Eindringen von Schimmelpilzen durch die Gelatineschicht hindurch zu verhindern, schliessen wir ausserdem den Rand des Deckgläschens noch mit Paraffin ab.

Das Sterilisieren bei höheren Temperatur der so vorbereitete Glasschalen ist nicht möglich weil dadurch das Deckglas sich lockern würde. Zu diesem Zwecke giessen wir in dieselben etwas 4 % iger Formalinlösung und lassen einige Zeit stehen. Darauf wird das Formalin ausgegossen, den zurückbleibenden Rest mit sterilem Wasser ausgespült und die Glasschale getrocknet, welche so bis zu ihrem Gebrauch zurückgestellt wird. In diesem Falle wird die Schale mit Alkohol ausgespült und der zurückbleibender Alkohol mit sterilem Wasser entfernt. Die Schale bleibt sodann stehen bis die Unterseite des Deckglases trocken ist; man kan diesen Trockenwerden beschleunigen indem man die Schale über die Flamme eines Mikrobrenners hin und her bewegt. An die Unterseite des Deckglases kann nun der gewünschte flüssige oder feste Nährböden in ein oder mehreren Tropfen angebracht werden. Eine Methode welche sich sehr bewährt hat ist das Anbringen eines Ringes aus Agar-Nährböden, während in der Mitte ein Tropfen Wasser mit den Pilz abgelagert wird. Auf den Boden der Schale giesst man etwas steriles Wasser. Bisweilen hat man auf diese Weise noch den Uebelstand, dass der Tropfen Nährflüssigkeit verdampft oder der Agar- oder Gelatine-Tropfen austrocknet. Besonders Gelatine hat oft das Bestreben einzutrocknen. Um dies zu verhindern stellt man am besten die Kulturschale in einen geschlossenen feuchten Raum.

DIE BOTRYTIS-KRANKHEIT DER PÄONIEN

VON

F. H. VAN BEYMA THOE KINGMA

In jedem Frühjahr findet man junge Pflanzen von Paeonia, welche von Botrytis befallen sind, einer Krankheit, worauf ich schon längere Zeit meine Aufmerksamkeit gerichtet hatte. Sind die jungen Knospen angegriffen, so zeigen dieselben dichte Rasen von bräunlichen Konidien, welche aber in den von mir untersuchten Fällen keine genügende Aehnlichkeit mit denen von Botrytis Paeoniae aufwiesen, um sie als zu diesem Pilze gehörig zu bezeichnen. Immer fand ich nur Botrytis cinerea oder der Cinerea-Gruppe nahestehenden Formen, worüber eine genauere Untersuchung noch aussteht.

Anfang Mai 1927 nun erhielt ich aus Aalsmeer (Holland), woher damals auch Oudemans sein Material bezogen hatte, einige junge Pflanzen der Päonie, welche nicht an den Knospen, sondern an den Stengeln unterhalb der ersten Blätter angegriffen waren. Hier waren die Stengel über einer Länge von 6—12 c.M. ganz durchfault, der Inhalt war in eine schleimige Masse übergegangen und an der Aussenseite befanden sich ausgedehnte Rasen von bräunlichen Konidien, woraus stellenweise etwas weisses Mycel hervorbrach. An den unteren Teilen der Stengel befanden sich in der Längsrichtung zahlreiche kleine schwarze Sklerotien von etwa 1 m.M. Länge. Nach oben hin nam die Krankheitserscheinung an Intensivität ab und konnte man beim Durchschneiden der Stengel das allmähliche Fortschreiten des Pilzes genau verfolgen. Das Mycel wächst hauptsächlich im Innern der Stengel. Offenbar lag

hier die von Oudemans [1]) beschriebene Botrytis Paeoniae vor. Da sich jedoch die Merkmale dieses Pilzes nicht mit der in der Literatur angegebenen Beschreibung völlig deckten, war ich gezwungen sämtliche Angaben über diese Botrytis durchzusehen, fand aber dabei derartige Unstimmigkeiten und Widersprüche, dass es angebracht erschien darauf näher einzugehen.

Oudemans hat ursprünglich den Pilz nach den drei, ihm von Prof. Ritzema-Bos zugesandten Pflanzen beschrieben. Er gibt als Sporengrösse 16—18 μ bei 7—7½ μ an und weist besonders auf die Konidienträger hin, welche eigentümliche, mit Stacheln versehene „Ampullen" besitzen sollen. Sklerotien hatte er bis dahin nicht vorgefunden.

Ritzema-Bos [2]) sagt wörtlich: „Jede einzelne Conidie aber ist länglich oval, 16—18 μ lang und 7—7½ μ breit". Auf den von ihm infizierten Pflanzen fand er bloss ein einziges Mal ein kleines Sklerotium.

Flora Patterson und Vera Charles [3]) fanden Botrytis Paeoniae auf Päonien in den Vereinigten Staaten. Die Sporengrösse wird in dem Artikel nicht erwähnt. Betreffs der Sklerotien sagen sie: Bemerkenswert war die Schnelligkeit mit welcher sich Sklerotien in den Kulturen bildeten. Diese Sklerotien, etwa 1 c.M. gross entstanden in 4—5 Tagen. Auf den Pflanzen waren die Sklerotien 1—1½ c.M. gross.

Whetzel [4]) findet mindestens zwei bestimmte Botrytis-Arten auf den Päonien, welche gleichartige Erscheinungen verursachen. Die eine Form bildet grosse Sklerotien, die andre sehr kleine. Letztere werden auch auf den Pflanzen gefunden und besitzen Stecknadelkopfgrösse. In einem persönlichen Schreiben an Prof. Dr. Joh. Westerdijk teilt Whetzel noch mit, dass nach seiner Meinung Botrytis Paeoniae in Amerika die am meisten vorkommende Art ist. Eine Sporengrösse erwähnt er nicht, doch gibt er an, dass die kleinen Sklerotien typisch für diese Botrytis-Art seien. Die andere auf Päonien gefundene Botrytis-Art möchte er in die Cinerea-Gruppe untergebracht haben.

Schliesslich fand ich bei Klebahn [5]) über Botrytis Paeoniae Folgendes:

„In meiner früheren Publikation ist nicht erwähnt worden, dass bereits Ritzema-Bos eine Botrytis auf Convallaria majalis beob-

achtet hat, die nach ihrem Aussehen und auf Grund von Kulturversuchen mit einer die Päonien schädigenden Botrytis identisch sein soll und von Oudemans als Botrytis Paeoniae beschrieben worden ist".

Und weiter: „Ich vermisse aber an dem mir vorliegenden Material *) die eigentümlichen Ampullen, die Oudemans bei Botrytis Paeoniae beschreibt und abbildet und mein Material bildet leicht und reichlich Sklerotien, während Ritzema-Bos an der Botrytis von Paeoniae nur einmal ein kleines Sklerotium erhielt. Versuche, den Maiblumenpilz auf Päonien zu übertragen hatten wenig Erfolg, so dass doch vielleicht noch einige Zweifel en der Identität der Pilze auf Paeonia und Convallaria zulässig sind".

Fasse ich diese verschiedenen Resultate und Meinungen zusammen, so ergibt sich folgendes Bild:

Oudemans hat zuerst die länglichen Sporen des Pilzes gefunden, er wird aber wohl keine 100 Sporen gemessen haben, wie es damals auch noch nicht üblich war und so die Abmessungen von Sporen angegeben haben welche nach seiner Meinung die typische Form am besten gezeigt haben. Auf derselben Weise komme ich bei meinem Material gleichfalls zu der Sporengrösse 16—18 μ bei 7—$7\frac{1}{2}$ μ. Ritzema-Bos hat selber keine Sporenmessungen angestellt, die Angaben von Oudemans aber anscheinend so aufgefasst als wenn jede einzelne Spore die angegebene Grösse hätte, was, wie wir weiter unten sehen werden, nicht der Fall ist. Flora Patterson und Vera Charles haben Botrytis Paeoniae überhaupt nicht in Händen gehabt, wie aus der Grösse der Sklerotien hervorgeht. Wahrscheinlich ist es eine der von Whetzel als zur Cinerea-Gruppe gehörigen Formen gewesen. Whetzel hat wahrscheinlich wohl die typischen kleinen Sklerotien von Botrytis Paeoniae gefunden. Jedoch lässt er sich weder über Sporengrösse noch Ampullen aus.

Meine eigenen Untersuchungen an dem Material aus Aalsmeer haben nun Folgendes ergeben. Die Grösse der Konidien ist verschieden. Manche sind länglich oval und besitzen die von Oudemans angegebene Grösse, andere sind mehr eiförmig bis oval und deshalb kürzer und breiter. Als Mittel aus 200 Sporenmessungen fand ich für die Länge 13,93 μ und für die Breite 8,42 μ. Wie

*) Botrytis-befallene Convallaria-Pflanzen.

oben schon erwähnt fand ich an den Stengeln der jungen Pflanzen zahlreiche kleine Sklerotien. Diese bilden sich auch in der Kultur, wo sie derartig miteinander verwachsen, dass ein Netzwerk entsteht, welches man am deutlichsten wahrnehmen kann, wenn man die Petrischale gegen das Licht hält. An den Konidienträgern fand ich aber ebensowenig wie damals Klebahn die von Oudemans beschriebenen Ampullen, wie ich auch danach suchte, bis ich durch Zufall die Lösung des Rätsels fand. Es zeigte sich nämlich, dass die Konidienträger an der Basis aufgeschwollen sind. Diese Aufschwellungen haben tatsächlich die von Oudemans angegebene Form. In einigen, zwar nicht in allen Fällen, konnte ich auch die „Stacheln" wahrnehmen, kurze rhizoidartige Ausstülpungen, in anderen Fällen war der Umriss mehr zackig. (Fig. 4). Oudemans muss also unerklärlicherweise die Unterseite der Konidienträger für die Oberseite angesehen haben, was um so leichter möglich sein konnte, als im Präparat mehrmals Konidien an den „Stacheln"-haften bleiben.

Hierdurch ist nun auch mit einem Schlage die Stellung der von Klebahn auf Maiblumen (Convallaria majalis) gefundene Botrytis aufgeklärt. In seiner Abhandlung: „Ueber die Botrytiskrankheiten und die Sklerotienkrankheiten der Tulpen, die Botrytiskrankheiten der Maiblumen und einige andere Botrytiskrankheiten, 1905, sagt er Seite 20—21:

„Ich beschränke mich darauf eine Abbildung mitzuteilen und dazu zu bemerken, dass die Konidienträger eine Dicke von 12—17 μ haben und eine Länge von über 2 m.m. erreichen können, dass ihre Farbe ziemlich stark braun ist und dass die Konidien etwa ebenso gestaltet und annähernd ebenso gross sind wie die von Botrytis parasitica, nämlich 11—15 μ lang und 7—9 μ dick". Diese Angaben stimmen nun genau mit denen für Botrytis Paeoniae überein, während die von Klebahn mitgeteilte Figur genau die Konidienträger mit der angeschwollenen Basis wiedergibt. Da das Fehlen der Ampullen, nach welchen Klebahn solange vergebens gesucht hat, nunmehr genügende Aufklärung gefunden hat, steht auch einer Annahme der Identität beider Pilze nichts mehr im Wege. Das einzige Bedenken wäre nur noch das von Klebahn mitgeteilte Misslingen der gemachten Versuche, den Maiblumenpilz auf Paeonia zu übertragen, doch möchte ich diesem Versuche

solange keinen besonderen Wert beilegen, als nicht über die Art der Ansteckung genauere Versuche vorliegen. Ausserdem ist eine künstliche Uebertragung von Botrytis durchaus nicht immer leicht und hat deshalb ein negativer Erfolg noch keine genügende Beweiskraft.

Es scheint mir nach alledem also angebracht, Botrytis Paeoniae von neuem zu beschreiben und lasse ich deshalb hier die vollständige Diagnose folgen:

BOTRYTIS PAEONIAE (Oudemans) van Beyma

Mycel endogen, zu den Spaltöffnungen heraus aufrechte Fäden entsendend. 12—30 μ breit, Zellengrösse 100—250 μ.

Konidienträger sehr zahlreich, zu einem gleichmässigen Lager sich zusammenschliessend, mit körnigem Inhalt, unten meist stark braun gefärbt, nach oben hin fast farblos werdend, an der Basis geschwollen und hier bisweilen (nicht immer) mehr oder weniger zackig oder mit kurzen rhizoidartigen Ausstülpungen besetzt, am oberen Ende dagegen keulig oder unregelmässig verbreitert und abgerundet.

Konidien in Köpfchen von 50—80 μ Durchmesser, länglich rund, oval oder eiförmig, von verschiedener Grösse, 10—22 μ lang (meist 12—18 μ, Mittel aus 200 Sporen 13,97 μ) und 7—9 μ breit (meist $7^1/_2$—10 μ, Mittel aus 200 Sporen 8.14 μ, mit feinkörnigem Inhalt, leicht gefärbt, in grossen Massen bräunlich.

Aeste 3—5, spiralig unter 45° abgehend, unverzweigt oder an der Spitze einmal oder mehrere Male geteilt.

Reinkulturen. Auf Bierwürze wächst der Pilz schnell an und bildet ein grauweisses spinngewebartiges Mycel. Später (nach etwa 14 Tagen) entstehen zahlreiche kleine Sklerotien, anfangs weiss, allmählig aber schwarz werdend. Die Fähigkeit Sporen zu bilden geht in der Kultur bald verloren, doch gelingt es mitunter durch wiederholtes Ueberimpfen auf sterilisierten Hyacinthenzwiebeln einige Konidienträger zu bekommen. Auf Möhre wächst anfangs grauweisses Mycel aus, welches aber nach dem Erscheinen der kleinen Sklerotien verschwindet.

Aus einem mehrere Jahre in der Kultur gezogenen Stamme unter

dem Namen Botrytis Paeoniae entwickelte sich ein Pilz, der weder Sporen noch Sklerotien mehr zu machen im Stande war, der aber ebenfalls von kranken Päonien stammte. Anscheinend geht also auf die Dauer auch die Fähigkeit Sklerotien zu bilden in der Kultur zurück.

Fig. I. Reifer Konidienstand von Botrytis Paeoniae. Vergr. $\frac{200}{1}$

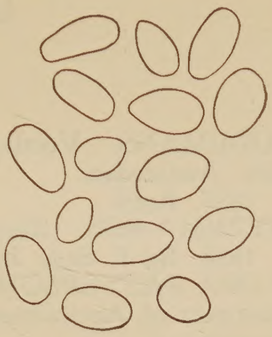

Fig. 2. Sporen von Botrytis Paeoniae Vergr. $\frac{650}{1}$

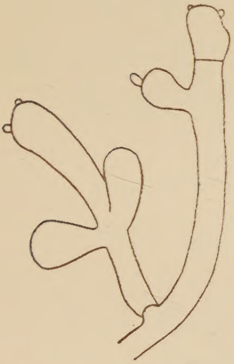

Fig. 3. Junge Konidienträger von Botrytis Paeoniae. Vergr. $\frac{650}{1}$

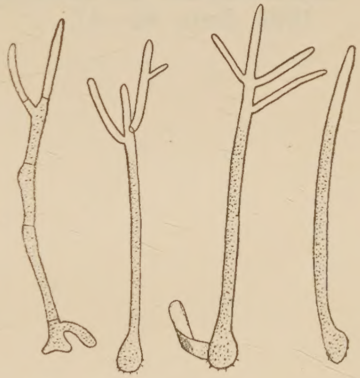

Fig. 4. Konidienträger von Botrytis Paeoniae mit den angeblichen „Ampullen". Vergr. $\frac{650}{1}$

LITERATUR

1) OUDEMANS. Verslagen der Kon. Akad. van Wetenschappen, Amsterdam.
1897, April.

2) RITZEMA-BOS. Zeitschrift für Pflanzenkrankheiten.
1898, Bd 8, Seite 264.

3) FLORA PATTERSON und VERA CHARLES. U. S. Department of Agriculture. Some fungous diseases of economic importance.
1910, Bull. 171, Seite 11—12.

4) WHETZEL. The American Florist, Vol. 44.
1915, Diseases of the Peony, Seite 611.

5) H. KLEBAHN. Weitere Untersuchungen über die Sclerotienkrankheiten der Zwiebelpflanzen.
1907, Seite 46—47.